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RATIO OF PHOSPHATE, NITRATE, AND POTASSIUM ON ABSORPTION AND GROWTH[1]

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(WITH NINE FIGURES)

In this paper are reported the results of experiments obtained in connection with a study of the effects of harmful soil constituents upon plant growth and upon soil solutions and fertilizer action. The results here reported are especially with reference to the growth made by wheat seedlings in culture solutions containing many different ratios of phosphate, nitrate, and potash, and in regard to the ratio of these constituents originally present and removed by the wheat seedlings in the course of the experiment. In these investigations solution cultures containing the three fertilizer ingredients, namely P_2O_5, NH_3, and K_2O, as calcium acid phosphate, sodium nitrate, and potassium sulfate, respectively, in all possible ratios of one, two, and three constituents, varying them in stages of 10 per cent, were prepared, the concentration being 80 parts per million in these constituents. The selection of the salts as carriers of phosphate, nitrate, and potash, and the statement of the results in terms of P_2O_5, NH_3, and K_2O, are in harmony with the practice in fertilizer work and, for the sake of simplicity, these designations are also retained in the present paper. The salts selected, it will be seen, are also carriers of calcium, of sodium, and of sulfate, and the three salts, therefore, are the best that could be selected for giving at the same time other needed constituents.

The culture solutions of these three salts contained in each case a total concentration of 80 parts per million of P_2O_5, NH_3, and K_2O,

[1] Published by permission of the Secretary of Agriculture.

but these varied, as above noted, in the ratio in which they were present. Wheat plants were grown in these various cultures and observations were made in regard to general development, the effect on root growth, and appearance. The green weight of the plants was taken at the termination of the experiment. The solutions were changed every three days and an analysis made, the phosphate, nitrate, and potash being determined, thus giving the concentration of these constituents and the ratio existing at the end of every three-day period for comparison with the original concentration and ratio. This changing of the solutions was kept up for twenty-four days, thus making eight changes. In this work the methods[2] devised in these laboratories for the determination of small amounts of such constituents rendered excellent service and point a way for their further use upon other problems in connection with the biochemical relationships of soils and plants, which have hitherto been impossible of attack. In the discussion and presentation of the results, the triangular diagram as used in physical chemistry was employed, and has proven very useful as a guide in the work for the systematic handling of the experimental details. The results can best be presented and interpreted by its means, and the method should prove very useful as a guide in other lines of experimental work where similar relationships are involved.

The use of the triangular diagram as a guide in the work

The number of solution cultures required in order to have all the possible ratios as outlined above is sixty-six. To bear in mind these ratios for three different ingredients, together with the ratios of the solutions after the plants had grown, and perhaps also the ratios of the material removed from the solution, a total of 594 numbers, is practically an impossibility, and it is readily seen that in order to discuss such a comprehensive experiment as this the material must be reduced to a workable basis, so that the various phases of the results can be kept in mind and the proper correlation and comparisons made. The triangular diagram as suggested by SCHREINE-

[2] SCHREINER, O., and FAILYER, G. H., Colorimetric, turbidity, and titration methods used in soil investigations. Bulletin 31, Bureau of Soils, U.S. Dept. Agric. 1906.

MACHER[3] in 1893 and again by BANCROFT[4] in 1902 has been of the greatest service to physical chemistry, where both theoretical and practical consideration of percentage composition of three component parts are concerned.

In the present investigation we have likewise to consider the three component parts of the fertilizer mixture; namely P_2O_5, NH_3, and

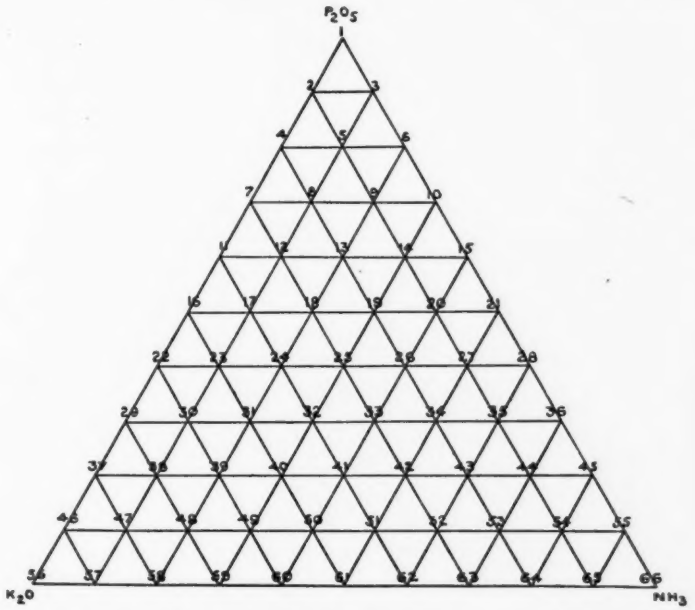

FIG. 1.—Showing the triangular diagram, with the points numbered, which represent the 66 culture solutions.

K_2O. It is possible, therefore, to represent any mixture of these three component parts in the triangular diagram. Such a triangular diagram is shown in fig. 1. It is an equilateral triangle in which the extreme points of the angles represent 100 per cent respectively of

[3] SCHREINEMACHER, F. A. H., Konzentrierung oder Verdünnung einer Lösung bei Konstanter Temperatur. Zeit. Phys. Chem. **11**:81. 1893.

[4] BANCROFT, W. D., Synthetic analysis of solid phases. Jour. Phys. Chem. **6**:178. 1902.

each of the ingredients, P_2O_5, NH_3, and K_2O, as shown in the diagram. Each side of the triangle is divided into ten equal parts, and lines are drawn connecting these points.[5] In the diagram, for the sake of ready reference, the intersections of these lines have been numbered. If we consider the line representing the base of the triangle, it is obvious that the point which represents 100 per cent K_2O (number 56 in the diagram) represents at the same time 0 per cent NH_3, and the point 100 per cent NH_3 (number 66) likewise represents 0 per cent K_2O. If we take a point half-way between these two points, for instance point 61, we have a mixture of the two salts in equal proportions; i.e., the fertilizer constituents represented by that point will be 50 per cent K_2O and 50 per cent NH_3. Similarly, point 16 represents 50 per cent K_2O and 50 per cent P_2O_5, and point 21 represents 50 per cent NH_3 and 50 per cent P_2O_5. If we take a point nearer to either of the corners, we will have a higher percentage of that ingredient and a correspondingly lower percentage of the other. For instance, at the point 59 the composition is 70 per cent K_2O and 30 per cent NH_3; at 29 it is likewise 70 per cent K_2O, but 30 per cent P_2O_5; at 64 it is 20 per cent K_2O and 80 per cent NH_3; at 45 it is likewise 80 per cent NH_3, but 20 per cent P_2O_5.

As stated above, points on the base line 56 to 66 represent mixtures containing no P_2O_5. The next line above this, namely 46 to 55, represents mixtures containing throughout 10 per cent P_2O_5, but varying amounts of the other two constituents. Similarly, the line 37 to 45 represents throughout 20 per cent mixtures of P_2O_5; line 29 to 36, 30 per cent mixtures of P_2O_5; and so on upward until point 1, the apex of the triangle, is reached, where the composition is 100 per cent P_2O_5, as already explained. Similarly, points on the line 1 to 66 represent 0 per cent K_2O; line 2 to 65 represents 10 per cent K_2O, but varying amounts of P_2O_5 and NH_3; and so on until at point 56 the composition is 100 per cent K_2O. Likewise, points on the line 1 to 56 represent 0 per cent NH_3; line 3 to 57 represents 10 per cent NH_3, but varying amounts of P_2O_5 and K_2O; and so on until at point 66 the composition is 100 per cent NH_3. It is obvious,

[5] Such diagrams for physical chemical work, giving still finer rulings, namely 100 to each line, can be purchased from the Cornell Co-operative Society, and were used in these investigations.

therefore, that any point within the triangle represents a mixture composed of the three constituents, its position in the triangle being determined by the composition of the mixture, namely the ratio of the three component parts, P_2O_5, NH_3, and K_2O. For instance, point 12, being on the 60 per cent phosphate line, represents this composition of P_2O_5, namely 60 per cent, and being at the same time on the 10 per cent NH_3 line, and the 30 per cent K_2O line, it represents 10 per cent and 30 per cent of these constituents, respectively. The composition of the mixture represented by this point is, therefore, P_2O_5 60 per cent, NH_3 10 per cent, K_2O 30 per cent; i.e., the ratio of these constituents in the fertilizer mixture is 60–10–30 or 6–1–3. Similarly, the point 34 represents the following mixture of the composition: P_2O_5 30 per cent, NH_3 50 per cent, K_2O 20 per cent, or a fertilizer ratio of 3–5–2.

For the sake of convenience in stating such ratios or percentage composition of the fertilizer mixtures in this investigation, the figures are always given in the order P_2O_5, NH_3, and K_2O, as shown above.

The triangle, therefore, represents single fertilizer constituents at the apices or vertices, mixtures of any two constituents along the boundary lines of the triangle, and mixtures of all three constituents within the triangle.

Solution culture methods

Before proceeding with a description of the general appearance of the cultures growing in solutions with the different fertilizer ratios, it may be well to describe briefly here the solution-culture method used in these experiments, as the manner of procuring a sufficiently large number of seedlings and of preparing physiologically pure water are important factors in carrying on this or allied investigations.

METHOD OF PROCURING UNIFORM SEEDLINGS

In the work under consideration, as well as in other work in progress in these laboratories, it is necessary to have a large number, often several hundred and sometimes thousands, of uniform seedlings; i.e., seedlings of the same age and equal in development and general vitality. The general principle on which this equal germination of

wheat seedlings is based has already been described by LIVINGSTON[6] and in former bulletins of this bureau.[7] The method consists in having a perforated disk, supported by ordinary corks in such a way that it will just float upon the surface of a pan of water. In the earlier work a wire gauze, which had been coated with paraffin so as to make virtually a plate of paraffin reinforced by wire, was used, holes being made in this plate by means of a hot wire. Perforated cork sheets, preferably paraffined, have also been used. In this case sheet cork about one-eighth of an inch in thickness is immersed in melted paraffin, and after removal from the paraffin this is allowed to harden in the air spaces of the cork. Holes are then made by means of a small cork borer so as to give a perforated plate. Both of these plates are open to objections. In the paraffined plate, when it is used continually, there is a tendency for the paraffin to split off from the wire, which is thus exposed to the action of the water and the roots; moreover, this plate is not easily repaired. The cork plate, on the other hand, shows considerable tendency to warp, is rather fragile, and is not easily kept sterile.

Instead of these plates there were used in these present experiments perforated hard-rubber sheets, thus overcoming the above objections to a considerable degree. These are prepared by cutting a circular disk of 305 mm. from vulcanized sheet rubber 3.2 mm. in thickness. By clamping several of these tightly together, preferably between layers of wood, small holes are drilled through the mass approximately 4.8 mm. in diameter and 5.0 mm. apart. Disks of this material float level upon the water when supported by corks, are not so subject to warping, and are readily cleaned and kept sterile. The corks are fastened to the under surface of the disk in four or five places on the circumference and in the center. For this purpose either rubber or wooden pegs or wire may be used; if the latter, it should be made either of iron or aluminum, never of brass or copper. The size of the corks is so gauged by trial that the disk is just supported on the surface

[6] LIVINGSTON, B. E., A simple method for experimenting with water cultures. Plant World **9**:13. 1906.

[7] SCHREINER, O., and REED, H. S., Some factors influencing soil fertility. Bulletin 40, Bureau of Soils, U.S. Dept. Agric. 1907.

of the water. After floating this perforated disk on the surface of the water, the wheat seed, which has been previously soaked for about two hours, not longer, is spread evenly over the surface. This method insures an even germination of the seed and has the advantage of keeping the seed and later the young seedlings just moist. The seeds are never submerged in the water, nor do they remain suspended dry above the water. The roots grow through the holes of the disk into the liquid below. This method of sprouting wheat seedlings is far superior to growing them in sand, since the seedlings are more uniform and the apparatus can be kept sterile much more readily than sand. Furthermore, for water culture purposes the seedling is readily removed by merely lifting it from the disk. In this way the roots or delicate root hairs suffer no injury whatever, while in removing seedlings from sand some injury would be unavoidable. Moreover, the seedlings are removed direct from water to grow in solutions, and thus have the advantage of germinating in a medium similar to that in which they are to be grown.

The water in the germinating pans may be distilled water, if desired, or where good tap water can be secured this may be used. The water in the pans is changed daily, or, during the warm weather, twice a day. Several hundred uniform seedlings can be procured from a single disk of the kind described. Whatever inferior plants occur are rejected. For the bottle culture work described in this bulletin, the seedlings were used when the plumule was about 2 cm. high and just ready to emerge from the enveloping sheet.

More recently disks of perforated aluminum have been used in this laboratory and have proven very satisfactory. These disks are floated by means of a raft of sealed glass tubing of such dimensions as is required to float the plates in the manner above described. Three lengths of tubing 102 cm. long, 35 mm. in diameter, with approximately a 1.5 mm. wall thickness, were sufficient to float six aluminum disks 30 cm. in diameter and 1.6 mm. in thickness. The lengths of sealed tubing and two glass rods 56 cm. long and 12 mm. in diameter are wired together into a raft. The entire arrangement is floated in a porcelain-lined iron tank. Fig. 2 shows this tank with seedlings at various stages of development.

NOTCHING CORKS AND MOUNTING SEEDLINGS

The bottles used in these cultures are made of flint glass, have a capacity of about 250 cc., a total height of 100 mm., an outside diameter of 70 mm. at the bottom, a neck about 20 mm. high, and a mouth 57 mm. in diameter. This bottle is stoppered by means of a soft, flat cork about 12 mm. in thickness and notched for holding the seedlings. The method of notching these corks consists in cutting ten vertical, triangular wedges from the circumference. Each wedge after being cut out is truncated, so that when replaced a small triangular opening, through which the plumule of the seedling will pass,

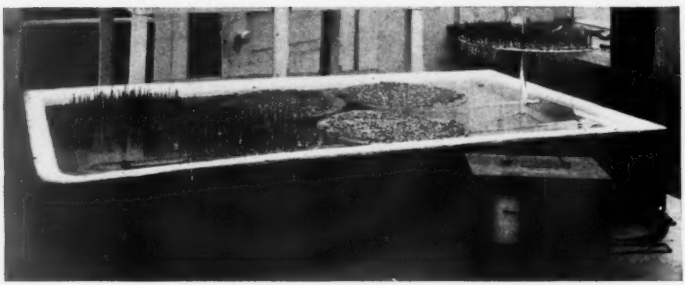

FIG. 2.—Method of germinating wheat seedlings on aluminum disks, which are floated in an enameled tank; disk to right elevated to show the plant roots.

is formed. This hole should be large enough to hold the seedling firmly and yet not bruise or injure it in any way by pressure. Around the circumference of the cork and in the upper half a groove is made sufficiently large to hold a small rubber band. After the wedges are inserted, the band keeps them in place and allows the cork with the seedlings to be handled readily and put into or taken out of the bottle without disturbing the plants. The seedlings are most easily inserted in the cork in the following manner: The cork with the ten wedges held in place by the rubber band is taken in the left hand and the seedling in the right hand. The plumule is pushed through the small, triangular opening, and then pulled up until the seed is close against the cork. When older seedlings, in which the leaves have unfolded

themselves, are to be used for an experiment, or where plants such as cowpeas are used, the wedge must be removed, the seedling put in place, and the wedge and rubber band replaced.

PREPARATION OF PHYSIOLOGICALLY PURE WATER

It is safe to say that ordinary distilled water, such as is commonly found in laboratories, is unsuited for culture experiments. This is due to a variety of causes which need not be entered into here, but which are discussed in a bulletin by LIVINGSTON.[8] Such water, while pure from a chemical and physical standpoint, is nevertheless not suited for such delicate indicators as plants, which are highly susceptible even to very minute quantities of toxic materials. Such water can be improved by distillation with strong oxidizing agents, such as potassium permanganate in alkaline solution or potassium bichromate in acid solution. The far simpler method already described by LIVINGSTON[8] has given very satisfactory results in this laboratory. In this method the water is purified by shaking it with a highly absorptive carbon black, which removes from the water any traces of injurious bodies it may contain. Not all carbon black possesses this property to an equal degree; the variety used in this laboratory is known as the "G Elf" brand, and is prepared on a commercial scale by burning natural gas and condensing the finely divided carbon on cooled surfaces. It is the same carbon black used for the purpose of decolorizing soil and plant solutions in this laboratory. For the present purpose the carbon black is thoroughly washed with distilled water as an added precaution, although it is not definitely known whether anything is thereby removed from it or not. The carbon black is then kept in this moist condition mixed with water so as to form a thin paste. About 10 cc. of this paste is added to each liter of water which is to be purified, and after shaking the mixture is allowed to stand for fifteen to thirty minutes and then filtered through an ordinary filter paper into a clean, hard-glass receptacle. Water treated thus is very satisfactory for the growth of plants in solution cultures.

[8] LIVINGSTON, B. E., Further studies on the properties of unproductive soils. Bulletin 36, Bureau of Soils, U.S. Dept. Agric. 1907.

General behavior of the cultures containing different fertilizer ratios

The plants in the different cultures prepared as described in the previous section show some marked differences almost from the very outset. After several changes of solution have been made, these differences become quite prominent. The most marked of these is

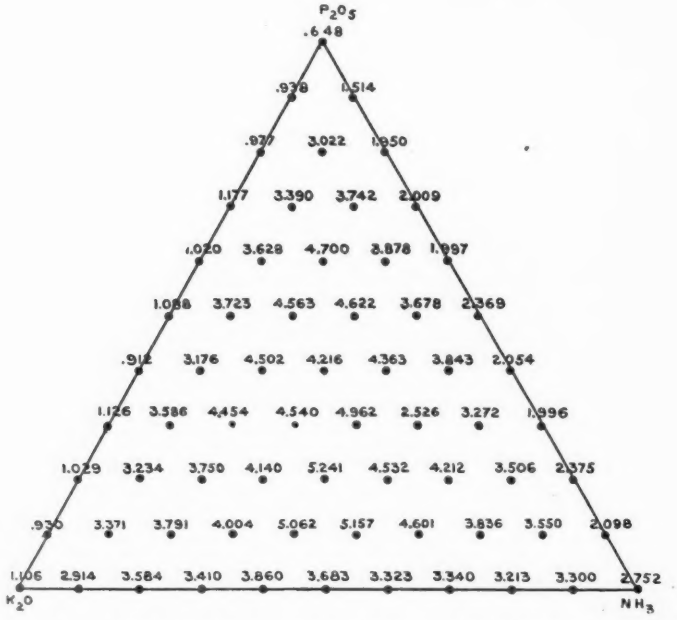

FIG. 3.—Green weight of 10 wheat plants grown in 66 cultures with different ratios of P_2O_5, NH_3, and K_2O.

that the solutions containing only one or two of the fertilizer ingredients, i.e., the entire periphery of the triangle, show a markedly less development of the plants than the cultures in the interior of the triangle wherein the three elements are combined. In other words, even the addition of only 10 per cent of the third element to mixtures of the other two produces a very appreciable increase in plant growth. This effect is most noticeable in the change from no nitrate to 10 per

cent nitrate. Of the single fertilizer ingredients, the nitrate culture is the best, and this is usually followed by the potassium culture, the phosphate culture being in general the poorest of the entire triangle. The cultures in the interior of the triangle do not show so marked a difference, although it is apparent that there is a tendency toward a rise in the middle of any line, this being displaced somewhat along some of the lines so as to give the effect of the highest and better region of growth lying in the middle of the lower part of the triangle; i.e., nearer to the potassium-nitrogen base. The set grew from February 25 to March 21. The green weights obtained in the sixty-six cultures are given in the triangular diagram shown in fig. 3. A more detailed discussion as well as grouping of these green weights, their correlation with the concentration of the solution, and the amount of nutrients removed will be given later.

As has already been stated, the solutions were analyzed every third day for the three component fertilizer parts, phosphate, nitrate, and potash, expressed as P_2O_5, NH_3, and K_2O. The original concentrations in these elements were in the sum total 80 parts per million. After the analysis the sum total of the three component parts was again calculated and the average concentrations of these three elements was again ascertained for the eight periods. The average concentrations will be found in the diagram in fig. 4. It is thus apparent that more of the essential ingredients were removed in the interior of the triangle; i.e., there was a greater absorption where all three were combined than where only two elements were present, and the least removal took place in those cultures where the single salts were present. It is also noticeable that in the solutions containing the three fertilizer elements the greatest removal occurred in that region of the diagram where the greatest growth occurred. A more detailed discussion of these results and their component parts will be given later in this paper.

Ratios of P_2O_5, NH_3, and K_2O, found in the various cultures

It might be said that in all cases the ratio in the final solution was never the same as the original ratio. The amount of change which had taken place, however, was markedly different in the different solutions and depended largely upon what the original ratio was. In

order to show this change in ratio of the fertilizer ingredients, the triangular diagram is of the greatest service, for without this it would be impossible to get an intelligent idea of what had occurred in the solution. In the diagram fig. 5 are given the original ratios of the fertilizer constituents, the ratios left in these solutions as shown by analysis, and the corresponding ratio of the removed constituents.

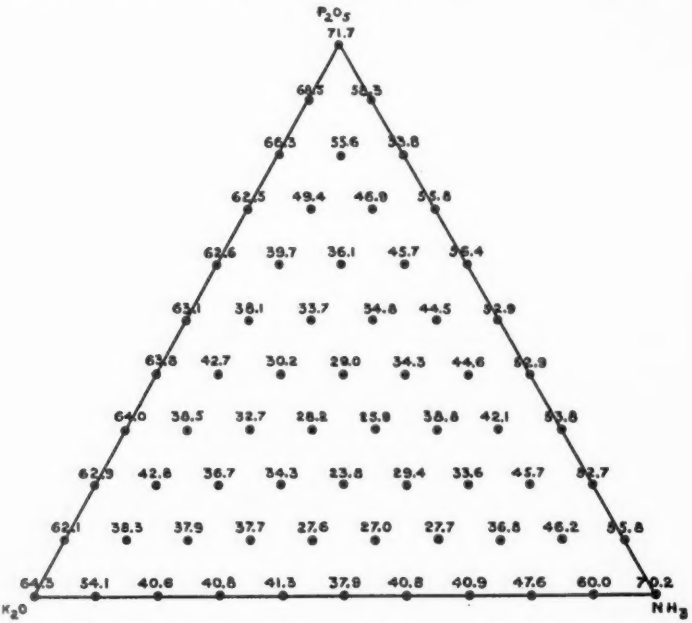

FIG. 4.—Showing the average concentration in parts per million of P_2O_5, NH_3, and K_2O of the solution after the growth of 10 wheat plants; concentration of the original solution was 80 ppm.

It may be shown mathematically that these three points lie upon a straight line and this is the case in the diagram.

The large dots in the diagram represent the original ratios corresponding to the scheme previously explained and given in fig. 1. The circles indicate the ratio left in the solution as shown by analysis. The other end of the line indicated by an arrow point shows the corresponding ratio of the removed materials. It must be borne in mind that

this diagram deals only with the ratios of the ingredients and not with the amounts that are present.

It is apparent that there is a decided tendency for these lines to converge toward a region somewhat below the center, as shown by the congregation of arrow points in this region. In other words, the solutions near this central area change least in their ratio, and the

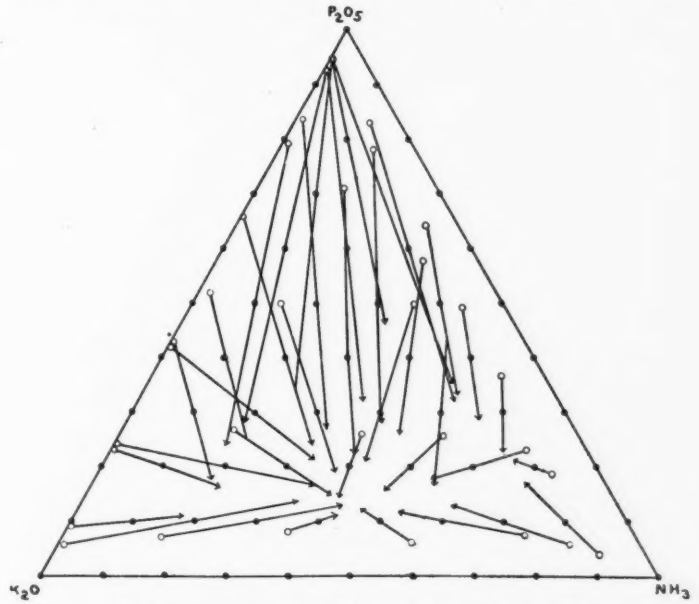

FIG. 5.—Showing the ratio of the original, the final, and the ratio of the loss of P_2O_5, NH_3, and K_2O from the culture solution: the dots indicate the ratio of the constituents in the original solution; the circles show the ratio of the constituents in the solution after growth; and the arrows show the ratio of the decrease.

farther the ratios were removed from this central area originally, the more were they altered in the course of the experiment. According to the diagram this area would seem to lie between the 10 and 20 per cent phosphate line, since the points on the lower line have moved in a direction opposite to that taken by the ratios in the upper part of the triangle. The data already presented show in general that the area of greatest growth occurred in this same region. It is in

this region of greatest growth, therefore, that the greater absorption took place with the least change in ratio; in other words, the solutions represented by this region offered the best environment for plant development and the best ratios for the absorption of plant nutrients.

Attention might also be called to the fact that in the ratios where the nitrate was low, there has been a movement to the no nitrate line, or at least so close to this line that it was impossible to plot them otherwise. After the comparatively small amount of nitrate was removed or reduced to a minimum, the point marking the ratio would have to move along the no nitrate base line in the direction either of the potash or of the phosphate, depending upon which was removed in the larger amount. The diagram shows that this movement of the ratio in the solutions was in nearly all cases toward the phosphate apex of the triangle. It is obvious that such a condition of affairs will cause a shifting of the lines connecting the ratios in the diagram, and that a similar state of affairs in the cultures on the low potassium line would produce a shifting of the ratio lines in the opposite direction. An examination of the diagram shows this has occurred, because the ratio lines in the upper part of the triangle show a divergence, giving a fanlike effect. In the case of the potash this divergence is also strongly noticeable, although the potash was never so completely removed as was the case with the nitrate.

The results of the experiments considered by periods

The very earliest periods, when compared with the later periods, show some differences in the ratio in which the three elements are removed, as has already been pointed out. Mention has been made of the fact that the ratios removed from the solutions along the lower phosphate line, namely, the 8 parts per million line of cultures, had a tendency to cause the ratio lines to turn from a point below this line to points above the line with increase in time. This general behavior of the cultures is very well shown by the three diagrams for the first, second, and third periods. The first diagram (fig. 6) shows the arrow points of the 10 per cent phosphate mixtures to lie below this line. The second diagram (fig. 7) shows some of the points above and some still below the line. In the third diagram (fig. 8) all but one arrow point lie above the line, and some arrow points of the 20 per cent

phosphate line are also noticed to have turned so far that some of them take a position even slightly above this line. There is a general tendency, moreover, for all of the points to lie somewhat higher for the other cultures, thus showing that this influence is probably common to all the cultures, though seen most strikingly in those containing small amounts, where an actual reversal takes place. This

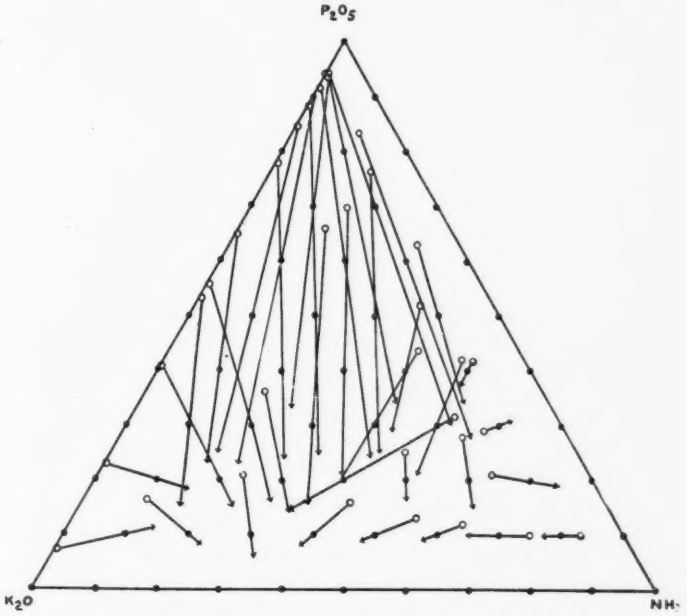

FIG. 6.—Showing the ratio of the original, the final, and the ratio of the loss of P_2O_5, NH_3, and K_2O from the culture solution in the first period.

general result may be interpreted to mean that the relative amount of phosphate removed in the early stages of the seedlings is not so great as it is subsequently, and the phenomenon can probably be correlated with the fact that in experiments where distilled water is used rather than these solution cultures, phosphate is excreted by the germinating seed and can be detected in the water in which germinating seeds or young seedlings bathe. Although the seedlings when used in these experiments have passed in the main through this

stage, the results show that there was a material lessening of the power to remove phosphate, probably due to the fact that the process of the phosphate absorption had not fully replaced the opposite function existing during germination and early growth.

The potassium absorption is also different from that in the later periods, although this is not so striking in the diagrams as in the case

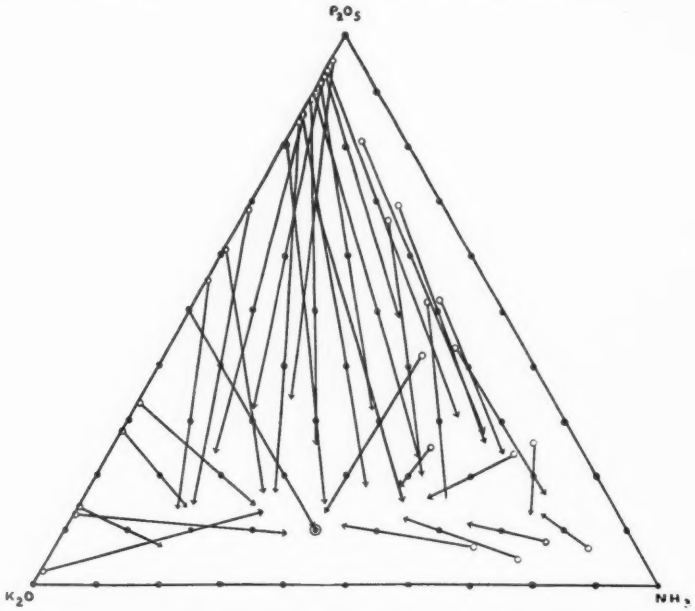

FIG. 7.—Showing the ratio of the original, the final, and the ratio of the loss of P_2O_5, NH_3, and K_2O from the culture solution of the second period.

of the phosphate. The average ratio of the removed material during these first three periods for all the cultures is 19 for phosphate, 39 for nitrate, and 42 for potassium; whereas the average for the five succeeding periods is 21 for phosphate, 44 for nitrate, and 35 for potash, thus showing that there is a tendency for a relatively greater potash and a relatively less phosphate absorption to take place during the earlier periods. So far as the influence of these fertilizer elements on growth is concerned, however, it is to be noticed that the nitrate has the

greatest effect, as is shown by the fact that the difference between the no nitrogen line and the 8 ppm. line is very great in the case of the nitrate, less in the case of the potash, and least in the case of the phosphate. As a rule, beyond the second or perhaps the third period the diagrammatic representation of the result is on the whole uniform, but is influenced undoubtedly by the conditions of growth during any

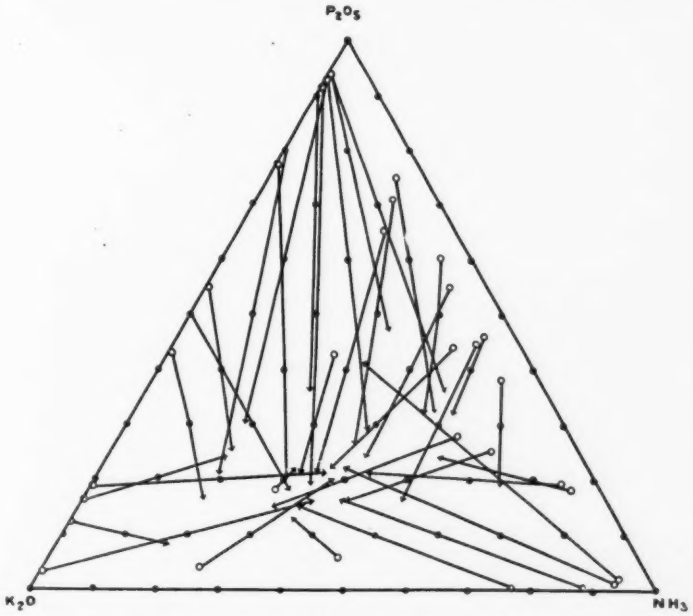

FIG. 8.—Showing the ratio of the original, the final, and the ratio of the loss of P_2O_5, NH_3, and K_2O from the culture solution in the third period.

period; in other words, by weather and other conditions, which is shown perhaps the quickest in the nitrate removal from the cultures.

Some remarks should here be made concerning the possible influence of bacteria. In the course of these experiments bacteria and other microorganisms were excluded so far as possible, but no special effort was made to maintain absolutely sterile conditions, inasmuch as this would have been a practical impossibility in an experiment on so large a scale. Moreover, it may even appear questionable whether

absolute sterility would be desirable. The bottles were sterilized before being used in making culture solutions for the various changes, the pans and other apparatus used in germinating the seed were sterilized from time to time, and corks used for the cultures were always clean and sterilized before use. Although all of these precautions were taken, it was of course not possible to exclude some microorganisms in such work, as the solutions were exposed from time to time to the air. There was in no case any excessive microorganic life noticeable. While bacteria and other microorganisms were present in the cultures to a slight and, under the conditions, unavoidable extent, it can hardly be said that their influence could have been large; that is, such influence as they had was probably so slight as to be negligible so far as the general and larger tendencies which are shown in this paper to exist are concerned.

Controls which were set up without having plants grown in them were found not to have changed their concentration or proportions of the constituents. Moreover, the various cultures support each other in their general tendencies. If the disappearance of nitrates were to be ascribed to bacterial activity, this should have shown itself all the more prominently as the age of the cultures increased. Such changes as were noticed from period to period might be ascribed to changes in the climatic conditions, thus still further affecting the plant's metabolism. This is very nicely illustrated in a series of preliminary experiments in which the solutions were changed every day instead of every three days, as was finally done. The diagram giving the results obtained on a clear day shows strikingly a relatively greater nitrogen removal under these conditions. The diagram giving the results obtained on the following day, which was cloudy and rainy, shows no less strikingly that relatively less nitrogen was absorbed on the cloudy and rainy day. It would be obviously unfair to conclude that under such conditions bacterial activities had been greater on the sunny day than on the following rainy day, especially as this result is in harmony with all observations on the removal of nitrate from solutions by plants. The processes of nitrogen utilization within the plant are known to be greater under conditions of better illumination. It is fair to assume, therefore, that this same general tendency held in all the other cultures, and that whatever

effect the bacteria may have had, it was slight in comparison to the activities of the plant itself under the conditions of this experiment.

Relation between growth and concentration of P_2O_5, NH_3, and K_2O found in the various cultures

The experimental results reported seem worthy of a closer analysis than that accorded them thus far. General tendencies as indicated

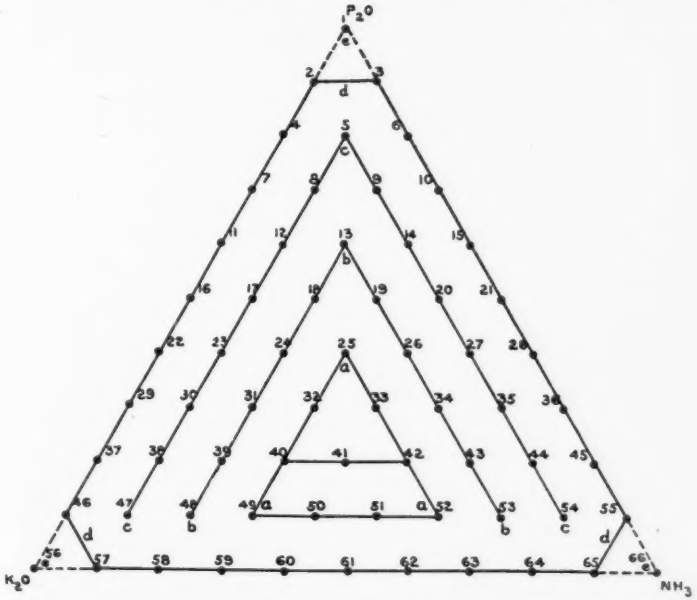

FIG. 9.—The arrangement of the culture solutions in groups: *a*, *b*, *c*, three fertilizer element groups; *d*, two fertilizer element group; *e*, one fertilizer element group.

by the individual cultures have already been pointed out, and these general impressions gleaned from the individual results can be better shown by a method of grouping. This method of grouping should be particularly valuable in showing the general tendencies, either in regard to plant growth or concentration differences connected therewith. Such a system of grouping, comparing different regions of the diagram, can be made in various ways.

The grouping shown in fig. 9 was made because by so doing the area of the greatest growth, which was always somewhat below the center of the triangle, will fall in these experiments into the central group. This central grouping is shown in the diagram as group *a*, the connecting lines showing the cultures included in this group. Group *b* takes in the cultures lying immediately outside of this central group. The same applies to group *c*, which is, however, farther removed from the central group. It will be noticed that these three groups contain the three fertilizer constituents. The next or fourth group contains all of the cultures in which two fertilizer elements occur, as shown by the lines. The fifth and last group consists of the sum of the single fertilizer elements. In handling the data, therefore, the average green weight in grams for each of these groups was determined, together with the average final concentration in the respective fertilizer constituents, or their combinations. In table I the average green weight and combined concentration of P_2O_5, NH_3, and K_2O is shown.

TABLE I

AVERAGE GREEN WEIGHT AND CONCENTRATION OF $P_2O_5+NH_3+K_2O$ OBTAINED IN DIFFERENT REGIONS OF THE TRIANGULAR DIAGRAM FIG. 9

(ppm. = parts per million)

CULTURE SOLUTIONS	NUMBER OF CULTURES INCLUDED	AVERAGE GREEN WEIGHT IN GRAMS	RELATIVE GREEN WEIGHT	AVERAGE P_2O_5, NH_3, AND K_2O		
				Original ppm.	Final ppm.	Percentage removed
Three fertilizer elements, group *a*	10	4.645	100	80	29.0	63.8
Three fertilizer elements, group *b*	11	4.120	88	80	35.0	56.3
Three fertilizer elements, group *c*	15	3.507	75	80	44.1	44.9
Two fertilizer elements	27	2.155	46	80	54.1	36.1
One fertilizer element	3	1.502	34	80	68.8	14.0
Three fertilizer elements, groups combined	36	4.010	..	80	37.0	53.8
All cultures combined	66	3.137	..	80	45.4	43.2

In the third column the relative green weight for the different groups is given, taking the results in group *a* as 100. From this it will be seen that the growth in group *b* is 88 per cent of that in group *a*, group *c* is 75 per cent; in the two fertilizer element group it is 46 per cent, and with the single fertilizer element the average result is only

34 per cent. The concentrations in these various groups are shown in the fifth column; the original concentration in all these cases was 80 parts per million of the combined constituents. In the first group, which was the one giving the greatest growth, the concentration was decreased from 80 to 29 parts per million, although, as has already been shown, the ratio of the three constituents in the solutions of this group suffered less change than did the ratio in the other groups. This can be seen from the diagram in fig. 5. In the second group the concentration was reduced to 35 parts per million; in the third group of the three fertilizer constituents to 44 parts; in the group of two fertilizer constituents it was reduced to 54 parts per million; and in the group containing one fertilizer constituent it was reduced to only 69 parts per million as an average.

For the sake of comparison, a group containing all cultures having the three fertilizer elements, as well as the combination of the entire group of sixty-six cultures, is given. The table also very clearly shows the markedly different effects produced on the green weight by a single fertilizer element and when these are used in combinations of two and of three, the results being approximately 1.5, 2.2, and 4.0, respectively, the higher group within the latter being as high as 4.6.

It will also be of interest to consider this same grouping in respect to the concentrations of the fertilizer elements individually. The number of cultures included in the group where two fertilizer elements were used was necessarily reduced, inasmuch as the ingredient in question did not occur in nine of the solutions, and the group of single fertilizer elements becomes, of course, reduced to only one culture, and for this reason has very little comparative value, although the result is usually decided enough to allow no doubt as to the general effect.

In the fifth column of table II is given the concentration of P_2O_5 found after growth, the original concentration being given in the fourth column for comparison. In the last column is shown the percentage decrease in the concentration of P_2O_5. This percentage decrease was in the first group 50 per cent, in the second group 37 per cent, and in the third group 27 per cent. In the combination of two fertilizer elements, one of which was phosphate, it

was 17.5 per cent, and in the phosphate by itself it was only 10 per cent. The actual decrease in concentration appears from the figures, however, to be strikingly uniform, varying in the different groups from 7 to 9 parts per million. This relationship of final to original concentration, however, is not properly shown by this form of grouping

TABLE II

AVERAGE GREEN WEIGHT AND CONCENTRATION OF P_2O_5 OBTAINED IN DIFFERENT REGIONS OF THE TRIANGULAR DIAGRAM FIG. 9

CULTURE SOLUTIONS	NUMBER OF CULTURES INCLUDED	AVERAGE GREEN WEIGHT IN GRAMS	RELATIVE GREEN WEIGHT	AVERAGE P_2O_5		
				Original ppm.	Final ppm.	Percentage removed
Three fertilizer elements, group *a*	10	4.645	100	16.0	8.0	49.7
Three fertilizer elements, group *b*	11	4.120	88	26.0	16.3	37.3
Three fertilizer elements, group *c*	15	3.507	75	34.0	24.9	26.9
Two fertilizer elements	18	1.531	33	40.0	33.0	17.5
One fertilizer element	1	0.648	14	80.0	71.8	10.3
Three fertilizer elements, groups combined	36	4.010	..	26.6	17.6	33.8

and can be much more satisfactorily handled, as will be done later, by grouping only such phosphate solutions as have the same original concentration. In the present table the original concentration is really an average of points varying, for instance, in the third group from 8 to 64 parts per million in phosphate content, giving the average concentration of 34 parts per million shown in the table.

TABLE III

AVERAGE GREEN WEIGHT AND CONCENTRATION OF NH_3 OBTAINED IN DIFFERENT REGIONS OF THE TRIANGULAR DIAGRAM FIG. 9

CULTURE SOLUTIONS	NUMBER OF CULTURE SOLUTIONS	AVERAGE GREEN WEIGHT IN GRAMS	RELATIVE GREEN WEIGHT	AVERAGE NH_3		
				Original ppm.	Final ppm.	Percentage removed
Three fertilizer elements. group *a*	10	4.645	100	32.0	9.8	69.4
Three fertilizer elements, group *b*	11	4.120	88	26.9	7.9	70.4
Three fertilizer elements, group *c*	15	3.507	75	22.5	5.8	59.9
Two fertilizer elements	18	2.721	58	40.0	22.8	43.1
One fertilizer element	1	2.752	34	80.0	70.2	12.2
Three fertilizer elements, groups combined	36	4.010	..	26.6	8.9	66.5

In table III is given the same character of results as have been given in the preceding section for phosphate, except that the concentration of nitrates is considered.

In the groups of two and single fertilizer elements, only those cultures containing nitrate are considered. In the three groups comprising the three fertilizer ingredients, the percentage decrease in nitrate is seen to be respectively 69, 70, and 60; in the two fertilizer element group it is 43; and in the single element, 12.

In table IV are given the results for the potash concentrations, similarly arranged and grouped as in the preceding tables.

TABLE IV

AVERAGE GREEN WEIGHT AND CONCENTRATION OF K_2O OBTAINED IN DIFFERENT REGIONS OF THE TRIANGULAR DIAGRAM FIG. 9

CULTURE SOLUTIONS	NUMBER OF CULTURES INCLUDED	AVERAGE GREEN WEIGHT IN GRAMS	RELATIVE GREEN WEIGHT	AVERAGE K_2O		
				Original ppm.	Final ppm.	Percentage removed
Three fertilizer elements, group *a*	10	4.645	100	32.0	10.8	66.2
Three fertilizer elements, group *b*	11	4.120	88	26.9	11.3	58.0
Three fertilizer elements, group *c*	15	3.507	75	22.9	9.6	58.3
Two fertilizer elements	18	2.212	48	40.0	26.1	34.8
One fertilizer element	1	1.106	24	80.0	44.3	44.6
Three fertilizer elements, groups combined	36	4.010	..	26.6	10.4	60.9

The percentage decrease in the cultures is for the three fertilizer elements in the three groups 66, 58, and 58, respectively; for the two fertilizer elements, 35; and for the single fertilizer element, 45.

Influence of different amounts of P_2O_5, NH_3, and K_2O, varying, respectively, from 8 to 80 parts per million

In the consideration of the results up to this point, no special discussion has been made in regard to the actual amounts of the individual fertilizer ingredients which were in the cultures. By reference to the original description of the diagram fig. 1 it will be seen, for instance, that there was a series of cultures in the line from number 46 to number 55 which contained 10 per cent of phosphate in the fertilizer mixture, or 8 parts per million of the culture solutions. Similarly, the line 37 to 45 contained 16 ppm.; the line 29 to 36, 24 ppm.;

and so on to the apex of the triangle. In the same manner, the line of cultures from 3 to 57 contained 8 ppm. NH_3 as nitrate; the line 6 to 58, 16 ppm.; and so on up to 80 ppm. NH_3 in culture 66. The line of cultures from 2 to 65 contained 8 ppm. K_2O; line 4 to 64, 16 ppm.; and so on up to 80 ppm. K_2O in culture 56.

It will be interesting to see what effect these different amounts of each fertilizer ingredient have had on the growth of the plant, and the removal of this constituent during the period of growth. In such

TABLE V

SHOWING THE RESULTS OBTAINED FOR P_2O_5 IN THE CULTURES CONTAINING DIFFERENT AMOUNTS OF P_2O_5 (PPM. = PARTS PER MILLION)

PERCENTAGE OF P_2O_5 IN FERTILIZER MIXTURE	PARTS PER MILLION P_2O_5 IN SOLUTION	AVERAGE CONTENT OF P_2O_5 IN FINAL SOLUTION, PPM.	AVERAGE DECREASE OF P_2O_5		AVERAGE GREEN WEIGHT	P_2O_5 DECREASE PER UNIT OF GREEN WEIGHT
			ppm.	percentage		
100	80	71.8	8.2	10.3	0.648	12.7
90	72	61.5	10.5	14.6	1.226	8.6
80	64	54.1	9.9	15.5	1.983	5.0
70	56	46.2	9.8	17.5	2.580	3.7
60	48	37.8	10.2	21.3	3.045	3.3
50	40	29.2	10.8	27.0	3.340	3.2
40	32	22.2	9.8	30.6	3.295	3.0
30	24	16.2	7.8	32.6	3.308	2.3
20	16	9.2	6.8	42.5	3.558	2.9
10	8	2.9	5.1	63.8	3.640	1.4
0	0	....	....	....	3.135	...

consideration it must always be borne in mind that there were present at the same time varying quantities of the other fertilizer constituents. For the purpose of such a comparison, the growth and concentration in the cultures along any of these lines has been studied and is presented in this section. With this in view all the results obtained in the various cultures in any one line were added together, and as the number of cultures naturally differ from line to line, the average was determined in each case for comparison, although this average is not intended to mean that all the cultures along this line were similar, for it has already been shown that in general the points in the middle were higher than those at the end of any one of these lines.

PHOSPHATE.—In table V are given the results obtained for P_2O_5 in the cultures containing different amounts of P_2O_5, varying from 80 to 8 ppm., as shown in the second column of the table.

The first column gives the percentage composition of the fertilizer mixture so far as the P_2O_5 content was concerned. In the third column is given the average concentration for the eight three-day periods in the experiment, and in the fourth column, the difference between this and the original concentration, thus giving the average decrease, which is expressed as a percentage in the fifth column on the basis of the original concentration. The sixth column gives the average green weight obtained along any of these concentration lines, and the next column gives the decrease in P_2O_5 calculated to the unit basis of one gram production of green plant. In other words, the decrease recorded in the fourth column has been divided by the green weight corresponding thereto in the sixth column. This gives, as it were, the rate of decrease in parts per million of the solution for each gram of green weight produced; but if it is desired to have the result expressed in terms of milligrams of P_2O_5 removed by each gram of green weight produced, the figures in the column must be multiplied by 2, since 250 cc. of solution were presented to the plants eight times; i.e., a total of 2000 cc. It will be noticed that the green weight steadily increased as the phosphate content decreased. It must be borne in mind, of course, that when the phosphate content decreases, there is a corresponding increase in the average content of both potash and nitrogen. In the last figure for the green weight, however, namely that in which the phosphate content became zero in the culture solutions, there is again a marked drop, although the potash and nitrogen content in these was higher than in any of the solutions above this in the table. It follows accordingly that a very distinct part was played by the phosphate in producing growth, although its maximum efficiency seems to be reached in these experiments in rather low concentration. Attention might also be called here to the fact that the concentrations of phosphate in soil solutions are always low, and relatively much lower than any of the other constituents here considered. The plant in its natural environment, therefore, has adapted itself to the occurrence of this constituent in weak solutions.

The increase in green weight shown by the table to be from 3.1 to 3.6 grams corresponded to an increase from 0 to 8 ppm. in the original P_2O_5 content. The further increase to 16 ppm. P_2O_5 has

produced no further increase in plant growth, and above this a decrease has even followed, which may be in part due to some direct influence of the phosphate solution, the carrier here used being calcium acid phosphate, and may also be due in part to the fact that the quantities of potash and nitrate are being decreased as the phosphate increases. This relation, however, has been discussed in a much more thorough manner where the ratios of these substances are considered, and the change in concentration which the solutions undergo, as shown in the diagram of ratio change in fig. 5, makes this matter very clear. It is there shown that the region of better growth is found in the lower part of the diagram, and this is also the region of most absorption and least change in the ratio. All these regions it will be noticed comprise solutions which contain low amounts of P_2O_5.

The average concentration of phosphate in the culture solution after growth, as shown in the third column, continually decreases. The actual decrease in the next column, however, shows that the greatest decrease in concentration of this element does not go with the greatest growth, but is found in the 40 ppm. phosphate solutions, increasing steadily from the 8 ppm. solution to this point, and then becoming practically constant up to the point where the other fertilizer elements entirely disappear and only phosphate remains, when it again drops slightly, the conditions for growth also being much poorer through this total absence of potash and nitrogen. The decrease when expressed as a percentage of the amount of material originally present shows that the removal is the more complete as the original concentration is lower. The last column in this section of the table, giving the decrease per unit of green weight, shows that equal weights of green plants cause quite unequal decreases in concentration, this change being least in the weaker solutions and greatest in the higher, thus indicating that more was removed by the plant in the higher concentrations than it could economically utilize under the conditions; i.e., the plant absorbed the material because it was there, although it apparently had already all that it could utilize economically. This is a fact consistent with field observations and ash analysis, where it is frequently noticed that the actual amount of mineral constituents is larger in the poorer plants.

NITRATE.—Table VI gives the results obtained for nitrate in the cultures containing different amounts of nitrate, similarly arranged and computed as in the phosphate table just discussed.

TABLE VI

SHOWING THE RESULTS OBTAINED FOR NH_3 IN THE CULTURES CONTAINING DIFFERENT AMOUNTS OF NH_3 (PPM. = PARTS PER MILLION)

PERCENTAGE OF NH_3 IN FERTILIZER MIXTURE	PARTS PER MILLION OF NH_3 IN SOLUTION	AVERAGE CONTENT OF NH_3 IN FINAL SOLUTION, PPM.	AVERAGE DECREASE OF NH_3		AVERAGE GREEN WEIGHT	NH_3 DECREASE PER UNIT OF GREEN WEIGHT
			ppm.	percentage		
100	80	70.2	9.8	12.3	2.752	3.6
90	72	54.2	17.8	24.7	2.699	6.6
80	64	42.0	22.0	34.3	3.046	7.2
70	56	32.8	23.2	41.4	3.169	7.3
60	48	24.1	23.9	49.8	3.492	6.8
50	40	18.3	21.7	54.2	3.685	5.9
40	32	12.0	20.0	62.5	4.166	4.8
30	24	6.1	17.9	74.6	3.852	4.6
20	16	0.5	15.5	96.3	3.893	4.0
10	8	0.2	7.8	97.5	3.156	2.5
0	0	....	....	....	0.995	...

The green weight column differs from the one discussed in the phosphate table in that the highest result occurs with the 32 parts per million NH_3 line, the numbers descending in each direction from his higher green weight. The change from 0 to 8 ppm. NH_3 is very marked in the green weight, which increases from approximately 1.0 gram to 3.2 grams, an even greater change than was noted in the phosphate table. The average decrease in concentration was in this case greatest between the limits of 32 to 64 ppm., the increase being gradual from the 8 ppm. solution up to the 32 ppm. solution, then being practically uniform up to 64 ppm., when it again declines. The figures in the percentage decrease column are on the whole much greater than those in the corresponding column in the phosphate table, thus showing a comparatively greater decrease of the nitrogen than of the phosphate in solutions of equal content of these elements, respectively. The last column, showing the rate of decrease per unit of green weight, shows the same general tendency for these figures to decrease with the decline in the original content of nitrogen in the solutions, as was noticed in the phosphate table, except that

the first two results, namely in the 80 and 72 ppm. solutions, are in this case lower than the following three or four entries, while in the phosphate table these two were the highest in the column. The conditions for growth with the phosphate in these two solutions, or groups of solutions, were very poor as compared with the rest of the results, while with the nitrate this difference in growth was not so apparent.

POTASSIUM.—In table VII are given the results obtained for potassium, the table being arranged and the results computed in the same manner as with those in preceding tables.

TABLE VII

SHOWING THE RESULTS OBTAINED FOR K_2O IN THE CULTURES CONTAINING DIFFERENT AMOUNTS OF K_2O (PPM. = PARTS PER MILLION)

PERCENTAGE OF K_2O IN FERTILIZER MIXTURE	PARTS PER MILLION OF K_2O IN SOLUTION	AVERAGE CONTENT OF K_2O IN FINAL SOLUTION (PPM.)	AVERAGE DECREASE OF K_2O		AVERAGE GREEN WEIGHT	K_2O DECREASE PER UNIT OF GREEN WEIGHT
			ppm.	percentage		
100	80	64.4	15.6	19.5	1.106	14.1
90	72	54.1	17.9	24.8	1.922	9.3
80	64	40.6	23.4	36.5	2.661	8.8
70	56	36.9	19.1	34.1	2.890	6.6
60	48	28.9	19.1	39.8	3.222	5.9
50	40	20.6	19.4	48.5	3.600	5.4
40	32	11.5	20.5	64.0	3.929	5.2
30	24	6.2	17.8	74.2	3.877	4.6
20	16	3.9	12.1	75.6	3.538	3.4
10	8	3.1	4.9	61.3	3.172	1.6
0	0	....	....	....	1.978	...

The green weight column is in its general tendency very similar to that observed in the nitrate table, namely that the 32 ppm. cultures give the highest green weight. The figures representing the average decreases and percentage decreases, on the other hand, correspond in trend more closely with those shown in the phosphate table, this similarity being especially shown in the last column giving the rate of decrease per unit of green weight, in which a regularly descending column of figures is seen. In the case of the green weight, the change from the 8 ppm. solutions to those containing no potash whatever was, as in the other cases, very marked, and this drop in green weight was also distinctly noticeable in the solution where potash alone was present. The results given in the three columns in the tables showing

the rate of decrease per unit of green weight are consistent in showing that relatively more was absorbed when more was present, although the plant does not seem to have been able to utilize this increase economically in its growth. This tendency is especially marked in the case of phosphate and potash, although it is also shown to an appreciable extent in the case of nitrogen.

As will appear in future publications, this general method of experimentation was used for the purpose of studying the effect of individual soil contituents and other organic compounds by using them in uniform concentration in all the cultures of a triangle. In these studies it was necessary to grow a control set without the constituent to be studied, so that the foregoing experiment was in this manner repeated a number of times, and the general results thus obtained were in harmony with those here recorded.

Summary

In this study the growth relationships and concentration differences were observed between solution cultures in which the phosphate, nitrate, and potash varied from single constituents to mixtures of two and three in all possible ratios in 10 per cent stages.

The better growth occurred when all these nutrient elements were present, and was best in those mixtures which contained between 10 and 30 per cent phosphate, between 30 and 60 per cent nitrate, and between 30 and 60 per cent potash. The growth in the solutions containing all three constituents was much greater than in solutions containing two constituents, the solutions containing the single constitutent giving the least growth.

The concentration differences noticed in the solutions were also very striking, the greater reduction in concentration occurring where the greatest growth occurred.

The change in the ratios of the solutions and the ratios of the materials that were removed from the solutions showed that where the greatest growth occurred, as above outlined, the solutions suffered the least change in ratio, although the greatest change in concentration occurred.

The more the ratios in these solutions differed from the ratios in which the greatest growth occurred, the more were the solutions altered

in the course of the experiment, the tendency in all cases seeming to be for the plant to remove from any and all of these solutions the material in the ratio which normally existed where greatest growth occurred. This did not actually occur in all cases, owing to the unbalanced condition of some of the solutions.

The results show that the higher the amount of any one constituent present in the solution, the more does the culture growing in that solution take up of this constituent, although it does not seem able to use this additional amount economically.

In the very early periods the ratio of phosphate absorption is low and the potash absorption high, although in final growth the greater response is obtained with nitrate, indicating relatively low phosphate requirement and high potash requirement of the seedling plant.

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CONTRIBUTIONS TO THE LIFE HISTORY OF WIDDRINGTONIA CUPRESSOIDES

W. T. SAXTON

(WITH PLATES I–III AND THREE FIGURES)

In a previous communication (**10**) the writer has pointed out certain peculiarities in this genus, and in the genus *Callitris*, which mark them off sharply from all other Cupressineae which have been investigated. Many phases of the life history have now been fully investigated in both genera, and in the main confirm the results previously published, although certain points require modification or correction. The investigation of both genera has been carried on simultaneously, the original intention having been to present the results in one paper; it has however been found that the differences between the two are more radical than was anticipated, and the present communication relates solely to *Widdringtonia*. A similar account of *Callitris* will be published very shortly (**11**), in which the results of both investigations will be discussed. The methods used have not differed materially from those previously employed.

1. The male cone

The sporophylls are arranged, like the leaves, in decussate pairs. Each sporophyll is peltate and bears four microsporangia[1] on the proximal side of its stalk. The output of spores from a sporangium is about 500. The structure of the sporophyll in a transverse section through the insertion of the sporangia is shown in fig. 1. The structure of the cone in radial longitudinal sections has already been figured (**10**, fig. 2). The anatomy of the sporophyll agrees exactly with that of *Callitris* and will be later described in that genus.

2. The female cone and the ovule

The young female cone consists of two spreading decussate pairs of sporophylls, each scale bearing seven or eight ovules on the proximal half of the upper surface, and close to the median ridge.[2] Each

[1] MASTERS (**6**) says 2–3.

[2] In *W. Schwarzii* only three ovules are borne on each scale.

megasporophyll is traversed by a large number of vascular strands and resin canals; near the top of the sporophyll the latter are evenly disposed all round the periphery, and inside each canal is a vascular bundle with the phloem on the side nearest the canal. On the lower (outer) surface of each scale is a small umbo.

The structure of the ovule before and shortly after pollination has already been described and figured[3] (**10**, figs. 1 and 3). The ingrowth of the micropyle-closing cells is not followed by septation in them, as it is for example in *Pinus*. A transverse section of the micropyle is shown in fig. 2, in which it may be seen that the opening is often not entirely closed by the ingrowth of these cells. This figure shows a most remarkable resemblance to a similar section of the micropyle of *Bennettites*, figured by LIGNIER (**6**, fig. 31), as pointed out to me by Professor SEWARD.

Some time after pollination the sporogenous tissue becomes evident. Seen in median section it appears as about 18 or 19 large cells with dense cytoplasm and large lightly staining nuclei, sharply marked off from the surrounding cells, which have vacuolate cytoplasm and smaller deeply staining nuclei. The position and structure of these cells are indicated in figs. 3 and 4. Their number[4] is about 64, possibly exactly that number, in which case they are not improbably formed by repeated simultaneous divisions of a single archesporial cell. It is however quite as likely that the number is somewhat less than 64, and that they have not a common origin from a single archesporial cell. They are all exactly alike when first differentiated, and their subsequent behavior indicates that they are spore mother cells, only one of which, however, is functional. Sometimes a single layer of tapetal cells may be seen a little later.

At a somewhat later stage, one of the central mother cells becomes a very little larger than the remainder, the nucleus also enlarges very slightly, and the wall becomes a trifle thicker than that of its neighbors. This is the functional megaspore mother cell. At about the same time the non-functional cells elongate somewhat, and certain peculiar

[3] The wings of the ovule were accidentally described (p. 166) as being only one cell thick. This is never the case, even close to the margin.

[4] Calculated on the assumption that the actual number is to the number seen in median section as $\frac{4}{3}\pi r^3$ to πr^2.

structures make their appearance at or near their ends. These structures appear as homogeneous dark brown bodies of various shapes, and may be conveniently called the "brown bodies" (fig. 5). That they are not produced artificially is clearly indicated by the fact that they are found only in the sporogenous tissue, and at first only in a very definite position at the two poles of the cells. Later they may be somewhat displaced from this position.

The only structures previously recorded as occurring in megaspore mother cells, with which the "brown bodies" might conceivably be correlated, are the "kinoplasmic centers" found in *Ginkgo* (CAROTHERS 1) and other gymnosperms. It seems unlikely, however, that the "brown bodies" are of this nature, especially as their existence has not been proved in functional mother cells. They seem to be entirely unstained by Delafield's hematoxylin, and show a marked resemblance in this respect, as well as in color and consistency, to certain nucleoli, although nucleoli usually stain deeply with this reagent. At about this time some of the cells in or near the central axis of the nucellus begin to break down or to elongate, no doubt in preparation for the rapid growth of the pollen tube and embryo sac (fig. 6).

3. Megasporogenesis

About a week after the events described have taken place the functional megaspore mother cell is found in synapsis (figs. 7 and 9), the surrounding cells retaining more or less their former character. No preparation has been obtained which shows either of the reducing divisions. Figs. 10 and 11 show a state of things rather difficult to interpret. The arrangement of the cells marked 1, 2, 3 in fig. 10, and the character of the nucleus of "1" as seen in fig. 11, at once suggest that "1" is the megaspore preparing for its first division, and that "2" and "3" are two sterile cells cut off in the heterotypic and homotypic divisions respectively. A comparison with figs. 12 and 13, which unquestionably represent two normal megaspores before the first division, indicates however that either this interpretation is not the true one or that the ovule was not normal.

In the preliminary account (**10**) a stage was figured which seemed to indicate a very large number of megaspores. Although every preparation of the same age showed an almost identical structure, it has

proved to be merely that characteristic of an ovule which is destined to be abortive.

It is uncertain whether three or four potential megaspores are formed, but it is quite certain that only one becomes functional. As shown in fig. 12, the surrounding cells begin to disintegrate so quickly that the other cells derived from the functional mother cell cannot be recognized. There is some indication that it is not the lowest megaspore which develops, as is usually the case, but no definite statement can be made. The wall of the mature megaspore is so thin that it can be distinguished only with great difficulty.

In fig. 13 a cell immediately above the megaspore shows one of the "brown bodies" very distinctly, and a part of this cell is drawn on a larger scale in fig. 14. Fig. 13 indicates that polarity may be established in the embryo sac before the first division of the megaspore nucleus, and many other preparations show the same, but by no means all (compare figs. 12 and 15).

4. The female gametophyte[5]

After the first division of the megaspore nucleus, the walls of the embryo sac and disintegrating mother cells can scarcely be distinguished. Their thickness has been unavoidably exaggerated in fig. 15. In the preparation figured (the only one showing a binucleate sac), no polarity is evident, nor are the nuclei arranged quite parietally. After the next division, however, the four nuclei are imbedded in (or rather protrude inward from) a very thin parietal layer of cytoplasm, and there is some indication of polarity, two nuclei being situated near the upper end and two near the lower end of the sac, the two pairs being symmetrically placed with regard to one another (fig. 16). This is distinctly interesting in view of the recently revived opinion that the embryo sac of angiosperms is derived from that of gymnosperms by a series of reductions, although it is to be noticed that certain "primitive" angiosperms do not show the polarity so characteristic of the vast majority. The growth of the embryo sac (prothallus) proceeds rapidly now toward the apex of the nucellus,

[5] As pointed out by COULTER and CHAMBERLAIN (2), the history of a gametophyte should start from the mother cell rather than from the spore, but practically it is more convenient to consider sporogenesis separately.

the apical part becoming very narrow and closely resembling the tip of an advancing pollen tube. The breaking down of certain cells in the axis of the nucellus, facilitating the upward growth of the prothallus, has already been referred to. The divisions of the nuclei are doubtless simultaneous until at least 64 are present (exactly that number having been counted in serial sections), and possibly until wall formation begins. Fig. 17 shows in outline the general structure of the nucellus at the time when 64 nuclei line the embryo sac. Fig. 18 is part of a tangential section of the prothallus after free nuclear divisions have ceased. The nuclei each contain a single nucleolus which can invariably be seen to be hollow.

Cell formation takes places as in most other gymnosperms, and has already been described and figured (**10**, fig. 9). A large number of nuclear divisions have been seen in the "alveoli," certain features of which strongly recall the peculiar divisions described by LAWSON (**4**) as occurring at this time in *Cryptomeria*. All the earlier phases (figs. 19 and 20) are quickly passed through, but that shown in fig. 21 is very persistent, the marginal fibers having almost the appearance of a cell wall. The wall between the nuclei, however, is always developed, but in cases where its width does not nearly equal the diameter of the cell, it is just possible that wall and fibers may disappear again and thus give rise to a binucleate cell. It has not been demonstrated, however, that binucleate cells ever originate in this way, and the chief interest of fig. 23 is in providing a connecting link between the very peculiar divisions giving rise to the temporarily binucleate cells of *Cryptomeria* and the ordinary mitotic divisions in normal conifer prothalli.

Very soon after cell divisions are complete, the archegonium initials make their appearance. These become considerably larger than the surrounding cells and their nuclei are a little larger (fig. 22). The position of the archegonia is somewhat variable, but usually two distinct types seem to be formed: (1) a single group of about ten or more, directly abutting on the swollen end of the pollen tube;[6] (2) one, or usually more than one, group of few or several archegonia (rarely a single archegonium) which are found near, but not abutting on, the upper part of the pollen tube, and deep-seated in the prothal-

[6] In the few cases where two pollen tubes reach maturity, a group of this kind is organized in connection with each tube.

lus (fig. 23). It is certain that neck cells are formed in the former (1), although they are very evanescent and much more difficult to demonstrate than in the archegonia of *Pinus*, for example; but they have not been demonstrated in the deep-seated archegonia (2), although it is not unlikely that this is merely due to the difficulty of differentiating them from other cells of the prothallus. In a recently published account of *Juniperus* (OTTLEY **9**), a case is described and figured in which a small deep-seated archegonium occurred outside the normal group. The figure leads one to suppose that no neck cell could be demonstrated, but no statement on this point is made in the text. After fertilization no neck cells can be found in either type of archegonium, which accounts for the suggestion made in my former paper (**10**) that neck cells are never formed in *Widdringtonia*. The neck, where present, consists of a single tier of four cells (fig. 23).

The central nucleus of the archegonium divides (in the first type of archegonium) simultaneously in the whole group (fig. 24), a conspicuous spindle being organized with long, slender, and considerably twisted chromosomes (fig. 24). The reduced number of chromosomes (six) may be counted fairly readily in this division. It is to be noticed in fig. 23 that although the central nucleus of every archegonium in the basal group is dividing, yet the nuclei of the other archegonia are still in the resting condition. The evidence that these do not divide is purely negative, but the possibility of the deep-seated archegonia being really archegonium initials only, must not be overlooked. The case shown in fig. 16 of my former paper (**10**) indicated that rarely a persistent ventral canal nucleus is formed, but out of scores of prothalli examined, containing thousands of archegonia, this is the only case in which such a state of things was clearly indicated. In no other preparation has the ventral canal nucleus been certainly identified, although the occurrence of the spindle which initiates its formation furnishes additional evidence that it must be formed. Doubtless it is normally very evanescent. From STRASBURGER'S (**12**) figures of the same nucleus in *Juniperus*, it would seem that it is by no means a conspicuous object in that genus, and although LAWSON (**5**) figures a large ventral canal nucleus in *Thuja*, it evidently disappears quickly, as it is not figured in the mature archegonium, and the same was found in OTTLEY'S recent paper on *Juniperus* (**9**).

The formation of binucleate and multinucleate prothallus cells has already been described. It is initiated at about this time, but nuclear divisions continue to occur during the earlier phases of embryo formation. Two such dividing nuclei are drawn in figs. 26 and 27.

The structure of the mature archegonium is much like that of other Cupressineae, with a large, centrally situated oosphere nucleus and a basal (or sometimes partly lateral) vacuole (fig. 28). The neck cells do not persist long, the archegonia of the basal group finally opening into the pollen tube.

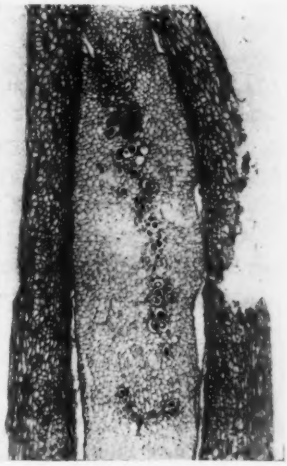

FIG. 1.—Microphotograph of a tangential section of a prothallus in which about 100 archegonia were present.

The total number of archegonia of both kinds organized in a prothallus varies considerably. Sometimes there are only about 25–30, usually about 40–70, and occasionally as many as 100. The microphotograph (text fig. 1) is taken from a tangential section of a prothallus in which about 100 archegonia were present. About 50 of these appear, cut transversely, in this section. A prothallus containing about 50 in all was sketched in my preliminary paper (**10,** fig. 15).

5. The male gametophyte

The earliest stages of the germination of the microspore have been already described, but the description will be briefly repeated here. The mature pollen grain is uninucleate and there is no evidence that any prothallial cells are formed. Thus the "pollen grain" is here synonymous with the "microspore." The earliest stage of germination usually consists in the formation of a solid outgrowth from the exospore, which applies itself closely to the surface of the nucellus, sometimes with a tendency to grow down between the nucellus and integument. About three pollen grains usually begin to germinate

in each ovule, but generally only one and never more than two reach maturity. A little later the solid outgrowth of the exine becomes hollow, and the intine then grows through it, forming the tube, which at once penetrates a short way into the nucellus, the single nucleus passing into the tube. Occasionally a tube is formed at once on germination.

The time during which the pollen tube remains uninucleate seems to vary a good deal, a tube with a single nucleus having been found associated with a four-nucleate prothallus (fig. 29), while in two or three cases a tube containing three nuclei was found in an ovule before the first division of the megaspore nucleus; one such case is shown in figs. 30 and 31. No differentiation of cytoplasm can be seen here around the upper nucleus, doubtless the body cell nucleus, but the preparation is noteworthy as the only one in which a difference in size could clearly be seen between the two sterile nuclei, that nearest the apex of the tube (the "tube" nucleus) being considerably larger. Fig. 32 is a detailed drawing of the tube, the position of which is shown in fig. 17. Its size is approximately the same as that of figs. 30 and 31, but the structure is somewhat different. The two sterile nuclei are precisely alike in every respect, as they are in every preparation in which they occur, except in the one noted above and in one abnormal tube referred to later. The body cell is here sharply demarkated from the surrounding cytoplasm in which the other nuclei are imbedded.

It should be mentioned here that the occurrence of only two nuclei in the tube at a stage somewhat later than this, as previously reported (**10**), has not been confirmed in any other preparations. It is curious that, in fact, the binucleate stage has never been certainly identified, as it is not improbable that the case just mentioned is really a three-nucleate tube, and that defective preparation is responsible for the appearance of only two. It may be added that the preparation there figured is from material collected very soon after the work was begun, and before the best fixing agents and oven periods had been satisfactorily ascertained. On the other hand, it may be that the uninucleate condition had persisted later than usual in this case, and that only two nuclei are actually present. In any event, it is certain that the two sterile nuclei must be cut off in somewhat rapid succession.

After they are formed, the pollen tube renews its activity and rapidly grows until it reaches the megaspore membrane. It is only rarely that the apex of the membrane is penetrated; more commonly the tube grows down a little farther before entering the prothallus. The entry is however almost invariably effected before the commencement of wall formation, the latter usually taking place only after the tube has taken up its final position. At the point where the pollen tube pierces the megaspore membrane, a slight constriction almost invariably occurs, while the tip of the tube dilates a little to form a kind of vesicle just within the prothallus. Preparations are often met with in which the contents of the tube are just opposite the constriction, indicating a certain amount of difficulty in passing this point. Inside the prothallus the wall of the tube becomes thicker (fig. 33). When the difficulty of passing this point is overcome, the tip of the tube rapidly advances to about, or a little beyond, the middle of the prothallus. During the whole later growth of the tube the individuality of the body cell is less evident than at the stage shown in fig. 32, and its cytoplasm may be quite indistinguishable from the rest of the tube cytoplasm. A case of this kind, in which all three nuclei are imbedded in a common mass of cytoplasm, has already been figured (**10,** fig. 10), and is not uncommonly met with (see also fig. 33).

Perhaps the most usual case is shown in figs. 34 and 35. Here the body cell is distinct, not only from the surrounding cytoplasm of the tube, but also from the cytoplasm in which the sterile nuclei are imbedded. With the triple stain, the cytoplasm of the body cell stains red, that in which the sterile nuclei are situated takes the orange, while the rest of the tube cytoplasm is a deep violet. In later stages the body cell increases very considerably in size. Its structure shortly before division has already been described and figured. Some uncertainty was expressed as to the fate of the two sterile nuclei. It was suggested that they were probably absorbed by and became indistinguishable from the cytoplasm. Later preparations have in the main supported this view, but sometimes these nuclei are recognizable in connection with a quite mature body cell, in a prothallus in which wall formation is complete.

Only a single preparation has been obtained showing the two male cells fully organized (figs. 36 and 37). Each cell is surrounded by

a definite wall (or membrane) and is more or less hemispherical. The cytoplasm is dense and very homogeneous, and in the center of each cell is a large nucleus with no nucleoli. The entire absence of starch in the male cells is noteworthy, as compared with other Cupressineae. In this preparation the two cells are just beginning to separate, and probably they would finally become rounded off as reported by LAWSON (4) in *Libocedrus*. In contact with one of these cells (see figs. 36 and 37) is a curious body which presents the appearance of a vesicle containing a number of small, deeply stained granules. No light can be thrown on the morphology or functions of this body. No trace whatever can be seen of the two sterile cells. Possibly their disappearance may be connected with the appearance of the vesicle, but this is merely conjecture.

6. Fertilization and embryogeny

The actual process of fertilization has not been seen in *Widdringtonia;* probably it agrees essentially with that in other Cupressineae as has been found to be the case in *Callitris*.

The first stage of the proembryo shows two free nuclei arranged lengthwise in the archegonium (fig. 38). The two nuclei are alike in size and structure. No starch is noticeable in the proembryo at this stage, but in all later stages seen, a considerable amount of starch is present. In later stages the structure has often been very difficult to interpret. Although a considerable number of preparations has been obtained, the sections have nearly always happened to be oblique. In two cases the following method has been successfully employed in order to get over this difficulty. Serial sections were drawn in ink on gelatin plates, the drawings being then placed together in sequence. If the thickness of the plates is to the thickness of the sections as the diameter of the drawing is to the diameter of the section, then a solid figure is obtained which represents accurately the object sectioned, the structure of which can be seen fairly clearly through the transparent gelatin. The gelatin plates can be easily stuck together by wetting them slightly.

Figs. 39 and 40 are reconstructed from serial sections. They show proembryos containing five and ten nuclei respectively. In fig. 39 delicate walls can already be seen between the nuclei, the

appearance of which indicates that they probably arose as cleavage planes. In fig. 41 a transverse section of the upper four nuclei is shown. The presence of kinoplasmic radiations (spindle fibers?) between these four nuclei indicates that probably they had a common origin and that after the first division of the oospore the basal nucleus remains undivided. It is evident that the early development of the proembryo does not show any resemblance whatever (after the first division) to that described by LAWSON (**5**) for other Cupressineae (*Thuja* and *Libocedrus*), and by OTTLEY (**9**) for *Juniperus*, where eight free nuclei are organized before walls are laid down.

Fig. 40 shows diagrammatically the structure of a ten-nucleate proembryo entirely filling the archegonium. It was not quite clear whether the walls extended to the upper three nuclei or not. The stages between figs. 39 and 40 are missing, and it is difficult to guess how the second form arose from the first. Probably there is some variation in the earlier divisions; compare, for instance, fig. 40 with the two proembryos figured in my preliminary account (**10**, fig. 16). Embryo development has already been briefly described.

Fig. 42 is a drawing of a germinating seed before the cotyledons are withdrawn from the testa. Fig. 43 shows the upper part of the same seedling after removal of the testa. Often the testa is carried up on the tip of one of the cotyledons in germination. The seedling structure (anatomy) of *Widdringtonia cupressoides* differs somewhat from that recently described by HILL and DE FRAINE for other species (**3**), and an account of it has already been published from this laboratory, together with a description of a remarkable twin seedling of the same species (MORRIS **8**).

In the plumular development a pair of opposite leaves succeeds the cotyledons, and is found in a plane perpendicular to that of the cotyledons. These leaves are followed by from about three to about ten alternating whorls of four leaves (text fig. 2). The structure and leaf arrangement of a tricotyledonous embryo have proved rather interesting. The three cotyledons are equal in size and have probably been equivalent in development and are succeeded by alternating whorls of three primordial leaves. This is shown in the photograph (text fig. 3). The transition region is longer than in the normal seedling, and only at its lower end is there any indication that the

three cotyledons are not all equivalent. Here two of the xylem groups grow somewhat smaller and approximate and finally join, thus giving the diarch root structure typical of the normal seedling.

7. Abnormalities

The tip of the pollen tube of fig. 23 contains a curious body cell which is shown more highly magnified in fig. 23*a*. Its appearance strongly suggests that it is not a normal body cell just after the division of the nucleus, but that during or immediately following the division disintegration of the nucleus has begun.

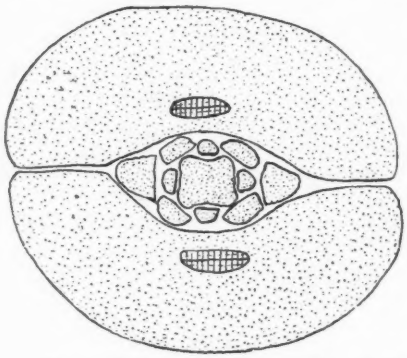

FIG. 2.—Diagrammatic cross-section of the cotyledons and plumule of *Widdringtonia*, showing arrangement of the primordial leaves in a dicotyledonous seedling.

In fig. 34 a slight irregularity is evident, inasmuch as a small fragment of the body cell nucleus has been constricted off and left just behind in the cytoplasm of the cell. It is barely possible that this may have been caused by defective preparation, but one other case has been seen in which a small papilla protruded in precisely the same direction from the body cell nucleus (not figured). Moreover, no such phenomenon has ever been seen by the writer in the case of any other large nuclei, either in *Widdringtonia* or any other plant.

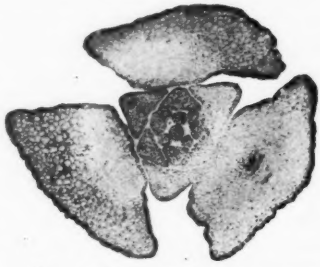

FIG. 3.—Photograph of a transverse section of a tricotyledonous seedling.

One very curious abnormal male gametophyte is shown in fig. 44. Here the sterile nuclei are in the swollen tip of the tube (which is surrounded by the usual group of archegonia), but the body cell has been left far behind. The nuclei are shown in more detail in figs. 44*a* and 44*b*. It is noticeable that the body cell does not exhibit such

a distinctive structure as is normally the case, while one of the sterile nuclei is clearly larger than the other and is also distinguished structurally from it. This also is unusual and suggests the possibility that this larger cell might have eventually taken the place of the tardy body cell.

The other two cases figured which seem to be abnormal are both taken from archegonia occurring in prothalli in which the pollen tube had lost its contents, but in which no proembryo was evident. Both might conceivably be taken to represent stages in the fusion of sexual nuclei. Fig. 45 shows an archegonium which was unhesitatingly considered to contain the sexual nuclei in contact, but on examination of these nuclei with a more powerful objective they were found to have the structure indicated in fig. 45*a*. The whole of the contents seems to consist of homogeneous and rather dense nuclear plasm except for the four nucleoli (?). Each of these nucleoli (?) consists of a membrane from the inside of which a plasmic strand has contracted on which are regularly arranged about six (three or four in optical section) very deeply staining bodies, of the shape and size figured. It is suggestive that the number of these bodies in each nucleolus (?) should be the same as the haploid number of chromosomes. It is also suggestive that two of these nucleoli (?), differing only in size, should be present in each of the fusing nuclei, if such they are.

Figs. 46*a* and 46*b* are two successive sections of the same archegonium nucleus. The structure is so strikingly different in the two that it is difficult to believe they are actually parts of the same nucleus; but the fact is clear. In each section all the deeply staining structures (chromatin granules? and nucleoli?) have been figured, whether or not they occur in the same optical focus.

Beyond the suggestion made above, I have no opinion to offer on these figures. Since they occur at such an interesting stage of the life history it seems desirable to place them on record.

8. General

Since much variation has been noticed among the Cupressineae in the time elapsing between pollination and fertilization, it is interesting to compare *Widdringtonia* in this respect. The chief point of interest is that although a very long period intervenes between

pollination and fertilization, yet there is no break in the continuity of development, such as probably occurs in those temperate conifers which are pollinated in one season and fertilized in the next. It is further interesting to notice that there are absolutely no fixed periods, so far as the writer can judge, when definite stages may be found. It may be not out of place to observe that these facts have made the working out of the life history very much more difficult than would otherwise have been the case, which must be the excuse for the gaps which still remain in the present account.

At the time of writing (November 1909), complete data are not available, but cones known to have been pollinated in January (or possibly early in February) of the present year are still far from ready for fertilization. They are still under observation and may probably be fertilized by the time this paper finally goes to press, in which case a postscript will be added.

Very young female cones may be found at any time, but soon die off unless pollinated. The time of appearance of the male cones is very variable. During the present year the writer has failed to find a single male cone, except in the early part of January. Nevertheless, recently pollinated ovules were collected at the end of June, so that there probably must have been male cones in the vicinity during May.

During 1908, male cones were collected in April and again in May (mature in both cases and separated by a full four weeks). A large number of trees were examined frequently and carefully during those months in 1909, without a single male cone being found. The following field notes of the different aged cones met with at one time and in one clump of trees may be of interest. About March 1 the following female cones were found: (1) very young; scales still widely open; pollinated (probably about one month previously); (2) medium size; still quite green; ovules with well-developed embryo sac; (3) full grown, brown, but with junction lines of scales still green; ovules with full-size embryo sac but no trace of cell formation; (4) full grown, uniformly light brown; integument discolored; young embryos; (5) dark brown, showing signs of dehiscence; mature seeds. It was afterward found that (3) could be segregated into two separate batches, in one of which fertilization took place about the end of April, in the other about the middle of September.

As stated in the introduction, it is not proposed to discuss the facts here reported until the corresponding facts in the life history of *Callitris* are published, when the two genera may conveniently be compared and contrasted.

Summary

The microsporophylls are arranged in decussate pairs and each bears four microsporangia.

The mature pollen grain is uninucleate.

The four equal megasporophylls are opposite and decussate.

About 64 megaspore mother cells are organized at the base of the nucellus, but only one is functional. Peculiar structures are noted at the poles of the non-functional megaspore mother cell.

The megaspore may show polarity before the first division of the nucleus, or when four nuclei are present. The divisions of the embryo sac nuclei are probably simultaneous.

Cell formation in the prothallus is normal in most respects, but certain peculiarities have been noted in the nuclear divisions which suggest comparison with *Cryptomeria*.

The archegonia are never situated at the apex of the prothallus, but in several groups organized in relation to the pollen tube and deep-seated in the prothallus. The lowest group abuts on the pollen tube; the upper groups do not, and may possibly represent archegonium initials. In the lowest group four neck cells are formed and a ventral canal nucleus is cut off. The total number of archegonia in a prothallus varies from about 30 to about 100.

The microspore nucleus remains undivided for a long time. In other respects the development of the male gametophyte is more or less normal, but the so-called "stalk" and "tube" nuclei are almost invariably exactly alike and tend to disappear completely in the mature pollen tube.

The proembryo completely fills the archegonium, but the arrangement of the cells is somewhat variable. Walls are formed when less than eight free nuclei are present.

The mature embryo has two (very rarely three) cotyledons.

The cells of the mature prothallus are all binucleate or multinucleate.

Stages in the life history are found not to correspond to definite seasons, and a long time elapses between pollination and fertilization.

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POSTSCRIPT (March 26, 1910).—Wall formation in the prothallus of ovules pollinated about January, 1909, is now taking place. Fertilization is likely to follow in the course of a few days. It may therefore be stated that an interval of fourteen or fifteen months will elapse, in this instance, between pollination and fertilization.

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[7] A considerable number of papers bearing on the subject were quoted in my former paper. It is not necessary to repeat the titles here, only those actually cited in the text being given.

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EXPLANATION OF PLATES I–III

All figures were drawn with Zeiss camera lucida, microscope, and lenses, except figs. 39 and 40 (see text). In all: *b*, "brown bodies"; *c*, micropyle closing cells; *d*, megaspore mother cells; *e*, functional megaspore mother cell; *f*, male cells; *h*, integument; *n*, nucellus; *q*, body cell nucleus; *p*, prothallus; *r*, resin cavity; *s*, sterile nuclei; *t*, pollen tube; *v*, vascular bundle.

PLATE I

FIG. 1.—Transverse section of mature microsporophyll, showing positions of four microsporangia, resin cavity, and vascular bundle. ×35.

FIG. 2.—Transverse section through micropyle of an ovule about same age as fig. 3; the formation of the "wing" has only just begun. ×205.

FIG. 3.—Median longitudinal section of a young ovule, showing position of megaspore mother cells. ×85.

FIG. 4.—Section through megaspore mother cells in an ovule similar to fig. 3. ×480.

FIG. 5.—Two non-functional megaspore mother cells, showing characteristic appearance of the "brown bodies." ×825.

FIG. 6.—Part of nucellus of an ovule slightly older than fig. 3, showing formation of schizogenous cavities and sliding growth of three cells. ×205.

FIGS. 7, 9.—Megaspore mother cells in synapsis. ×825.

FIG. 8.—Position of mother cell of fig. 7 in relation to surrounding cells. ×205.

FIG. 10.—Sporogenous cells at a somewhat later stage (see text). ×500.

FIG. 11.—Cell 1 of fig. 10. ×825.

FIG. 12.—A functional megaspore and some of the surrounding cells. ×480.

FIG. 13.—A functional megaspore and one of the surrounding cells. ×480.

FIG. 14.—Part of upper cell in fig. 13, more highly magnified. ×1150.

FIG. 15.—A binucleate embryo sac showing disorganization of other sporogenous cells. ×360.

FIG. 16.—A four-nucleate embryo sac and the disorganizing mother cells surrounding it. ×205.

FIG. 17.—Longitudinal section of nucellus, showing position of pollen tube and embryo sac when 64 nuclei are present in the latter; the region occupied by disorganizing spore mother cells is shaded. ×35.

PLATE II

FIG. 18.—Some prothallus nuclei in tangential section of the sac, showing the hollow nucleoli. ×825.

FIGS. 19–21.—Three dividing nuclei in the "alveoli." ×1240.

FIG. 22.—Archegonium initial. ×205.

FIG. 23.—Part of a longitudinal section of a mature prothallus, showing the pollen tube, the basal group of archegonia, and three accessory archegonia; the nuclei of all the basal archegonia are dividing. ×120.

FIG. 23*a*.—The body cell of fig. 23. ×825.

FIG. 24.—Part of one of the archegonia of fig. 23, showing the spindle concerned in cutting off the ventral canal nucleus. ×825.

FIG. 25.—The neck of an archegonium in transverse section. ×205.

FIGS. 26, 27.—Dividing nuclei in the prothallus cells. ×1500.

FIG. 28.—Mature archegonium; the position of the pollen tube is indicated above the neck. ×360.

FIG. 29.—Young pollen tube; only a single nucleus is present. ×480.

FIG. 30 —Tip of young pollen tube showing three nuclei. ×825.

FIG. 31.—Part of nucellus, showing position of the tube of fig. 30. ×50.

Fig. 32.—Young pollen tube with body cell and two sterile nuclei; the position of this tube is shown in fig. 17. ×825.

PLATE III

FIG. 33.—Tip of pollen tube just entering the prothallus. ×205.

FIG. 34.—Tip of pollen tube after growth of the tube has ceased; the sterile nuclei with their surrounding cytoplasm form a distinct cell; a small fragment of the body cell nucleus has separated off. ×205.

FIG. 35.—The body cell nucleus of fig. 34. ×825.

FIG. 36.—Tip of mature pollen tube, showing position of male cells and the relation of archegonia to the pollen tube. ×205.

FIG. 37.—Male cells of fig. 36. ×480.

FIG. 38 —Part of a transverse section of a prothallus, showing an archegonium containing two free nuclei, the daughter nuclei of the oospore. ×360.

FIG. 39.—Diagrammatic longitudinal section of proembryo with five nuclei; reconstructed from serial sections. ×170.

FIG. 40.—Similar section of proembryo with ten nuclei; reconstructed from serial sections. ×230.

FIG. 41.—One of the sections from which fig. 39 was constructed, slightly oblique, showing the upper four nuclei and the cleavage planes (?) between them. ×200.

FIG. 42.—Young seedling. ×1.5.

FIG. 43.—Cotyledons of same. ×3.7.

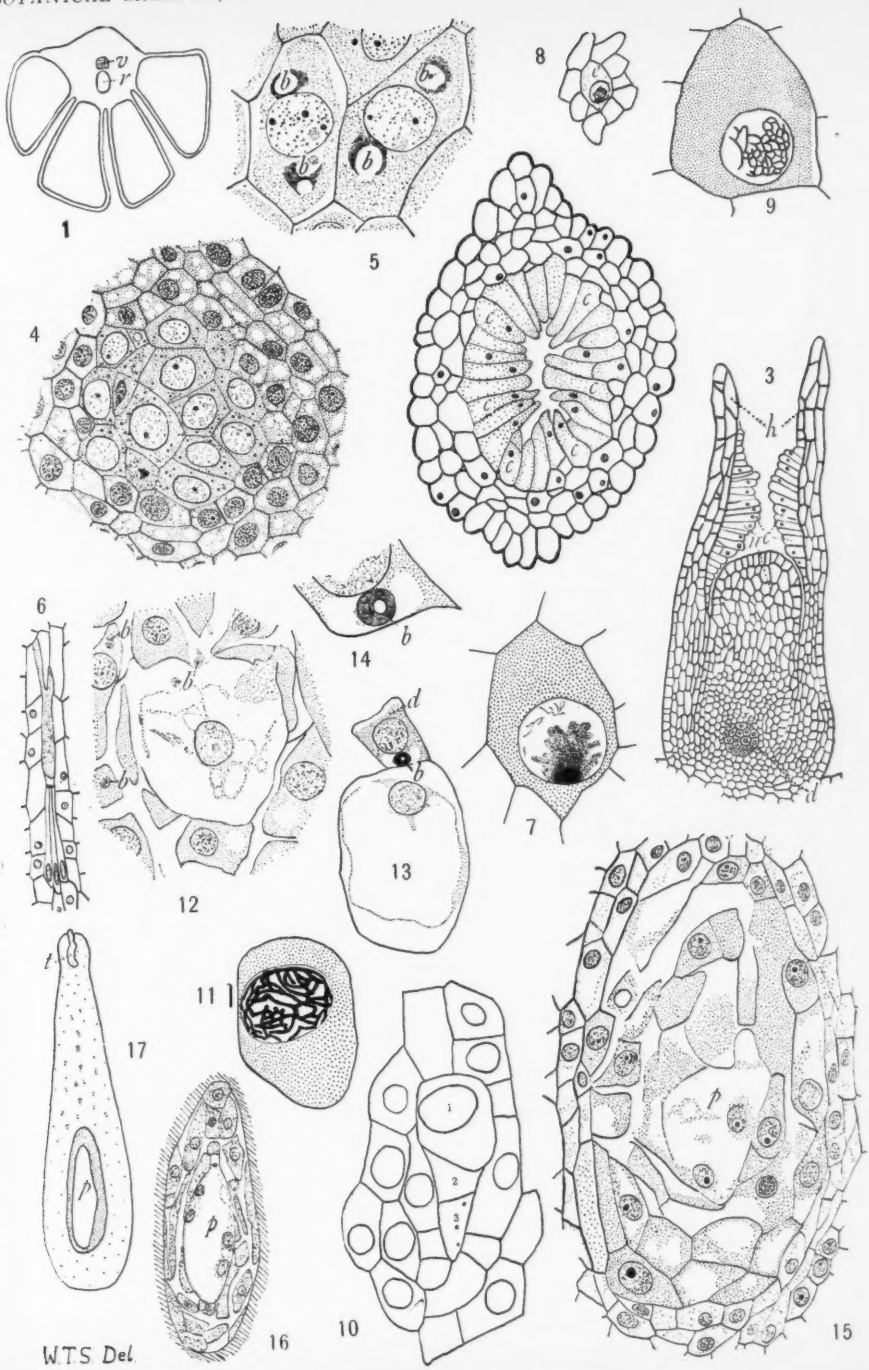
FIG. 44.—Abnormal pollen tube (see text). ×50.

FIGS. 44*a*, *b*.—Nuclei of fig. 44. ×205.

FIG. 45.—Abnormal archegonium. ×205.

FIG. 45*a*.—Part of fig. 45 (see text). ×825.

FIGS. 46*a*, *b*.—Two adjacent sections of the nucleus of an abnormal archegonium. ×825.



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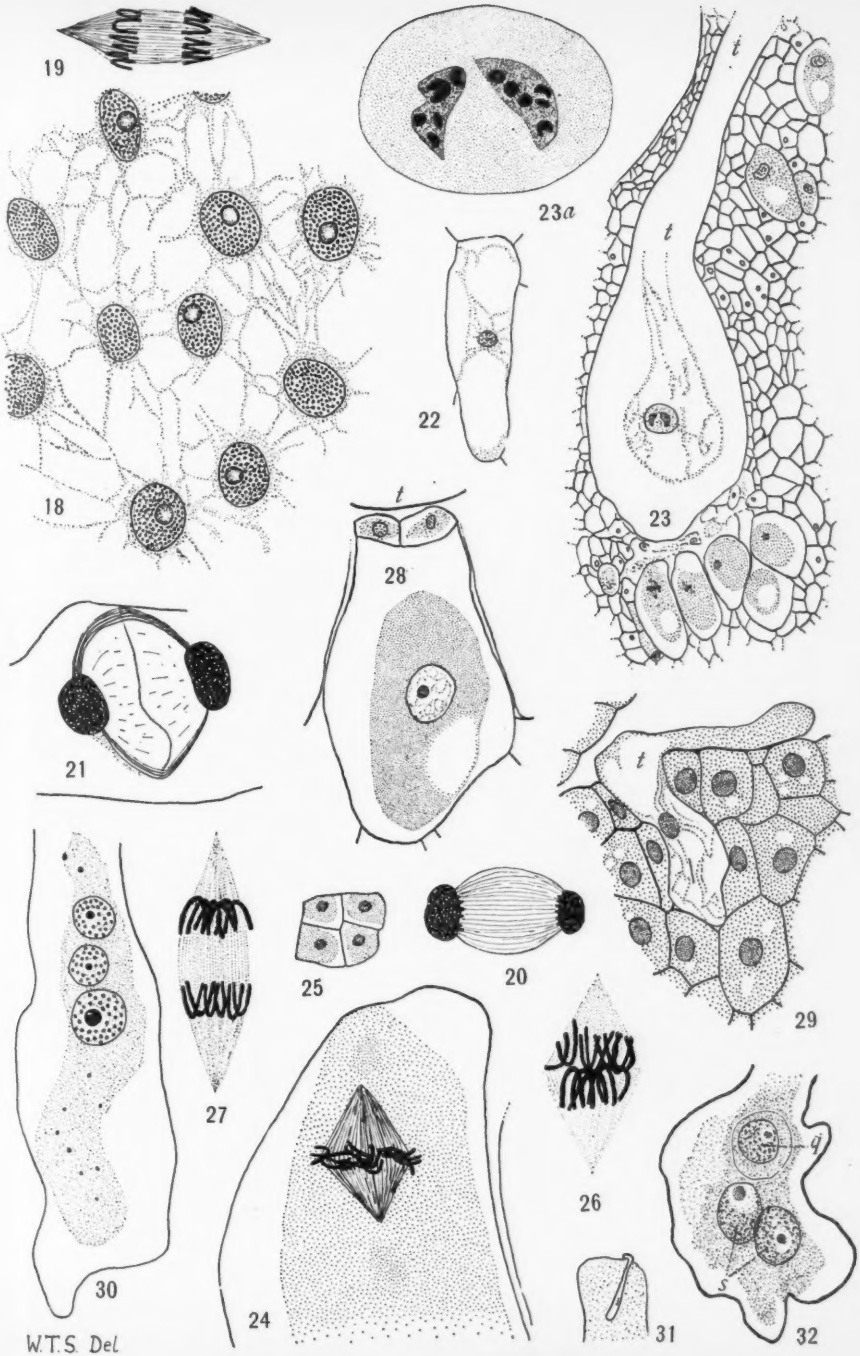

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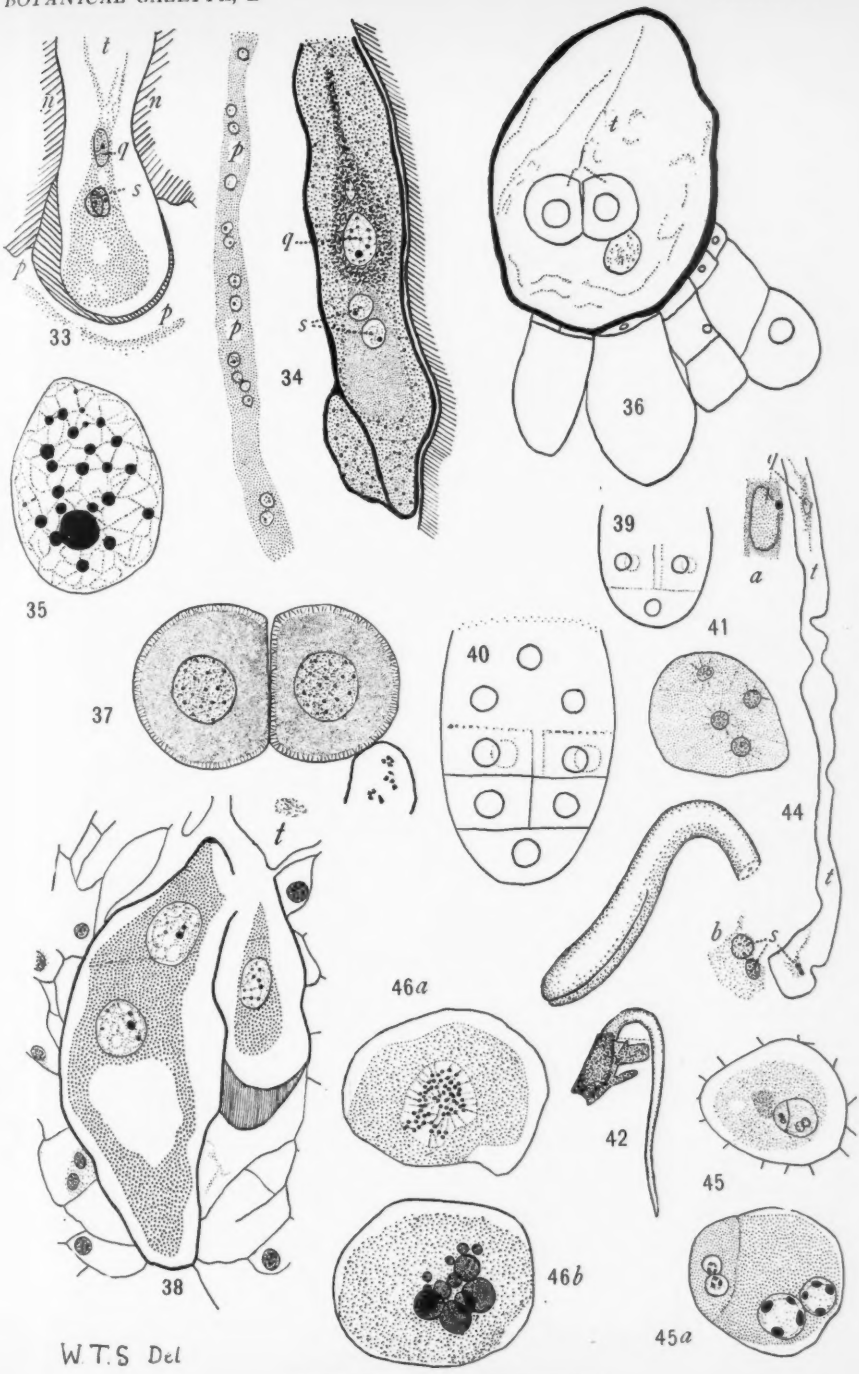

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SOME PECULIAR FERN PROTHALLIA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 137

LULA PACE

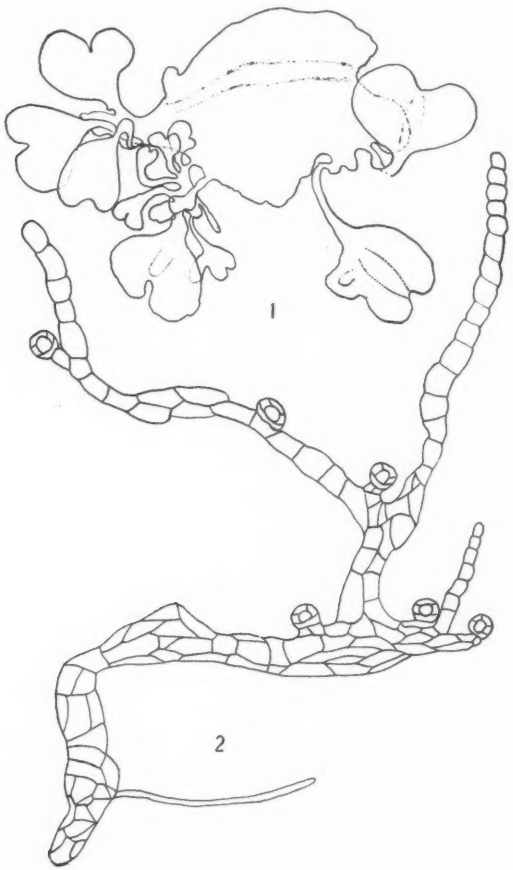
(WITH ELEVEN FIGURES)

In September 1906 Miss S. M. HAGUE sent me some fern prothallia from a swamp in northern Indiana, where they were growing luxuriantly on rotten wood. Pieces of the wood were put into a glass jar, which was covered and placed on a table about seven feet from a window with a southeast exposure. They were kept very moist, yet watered with care, so as not to allow any water to get on the plants and thus cause fertilization. In spite of this precaution, however, enough moisture probably collected on the plants to permit fertilization occasionally, for sporophytes developed at intervals. Some of these, as well as many of the gametophytes, were used in class work. A few of the sporophytes are about 7 cm. high. They resemble SLOSSON'S figures of *Dryopteris spinulosa intermedia* (*Aspidium intermedium* Muhl.). Certain peculiarities found in this material seem worth describing. Some of the gametophytes are still growing and will be watched for further developments.

Gametophytes

The prothallia were of the typical heart-shaped form, the larger ones being about 5 mm. long. Those on which sporophytes have not developed have continued to grow, and many spores, which had been lying for a long time in the rotton wood, have germinated. The old prothallia have grown to unusual size and taken queer shapes, many of them being 15 mm. long, a few 23 mm., and one even measuring 37 mm. The most striking thing about them, however, is the peculiar forms developed. A hasty observation of one of these often gives the impression of a number of prothallia near together and overlapping, for they often branch, apparently from any part of the plant, as in fig. 1, which is a diagram of one of the simpler cases. At one point near the margin of another plant 21 of these branches were counted, all 1–1.5 mm. long, and each bearing 6–10 antheridia. In

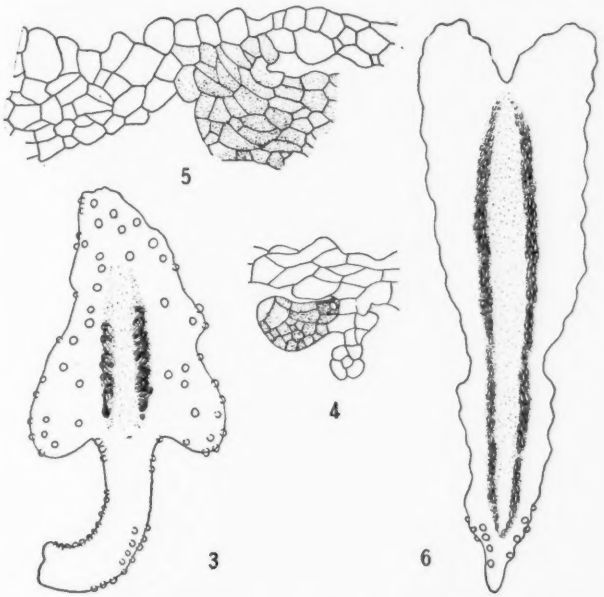
some cases filamentous processes grow out (fig. 2); this figure was made from fresh material, and the cell walls may not be accurate in



FIGS. 1, 2.—Fig. 1, diagram of one of the simpler prothallia with several gametophytic branches; fig. 2, sketch of a filamentous process with antheridia, from living material.

every case. Occasionally a cylindrical process was seen, as described by LANG (**2**), but it might possibly have developed into the ordinary form, for some of the branches have slender bases. In several

instances this outgrowth is from the apical region, as shown in the diagram (fig. 3), which represents a small prothallium 5 mm. long. The outgrowth has antheridia along the margins, but shows no archegonia; these were present, however, on the main prothallium.



FIGS. 3–6.—Fig. 3, prothallium 5 mm. long, with apical process bearing antheridia but no archegonia, which are found on the main prothallium; fig. 4, section through a very young gametophytic branch with apical cell; the branch is at the edge of the section and near an archegonium, the cross-section of the neck of which is shown; fig. 5, section through the basal part of a gametophytic branch, showing its relation to the main gametophyte; fig. 6, an antheridial prothallium 23 mm. long, with many archegonia but no antheridia except in the basal region.

Sections of this prothallium show nothing unusual in structure or cell contents.

A section of one of these branches at a very early stage shows its relation to the main prothallium (fig. 4). It comes from the edge of the archegonial cushion, the section showing also the neck of an adjacent archegonium. So far as the apical region is concerned, it

looks like a normal gametophyte. The basal part of a somewhat older branch is shown in fig. 5, which is broader and does not seem so closely related to archegonia, although near the cushion. Apparently any cell or group of cells may be rejuvenated and initiate this branching. The cells in the neighborhood of fig. 4 are very large for cells in a position so near the apical region, and they contain unusually large food bodies, apparently oil globules; this may be a condition that leads to branching.

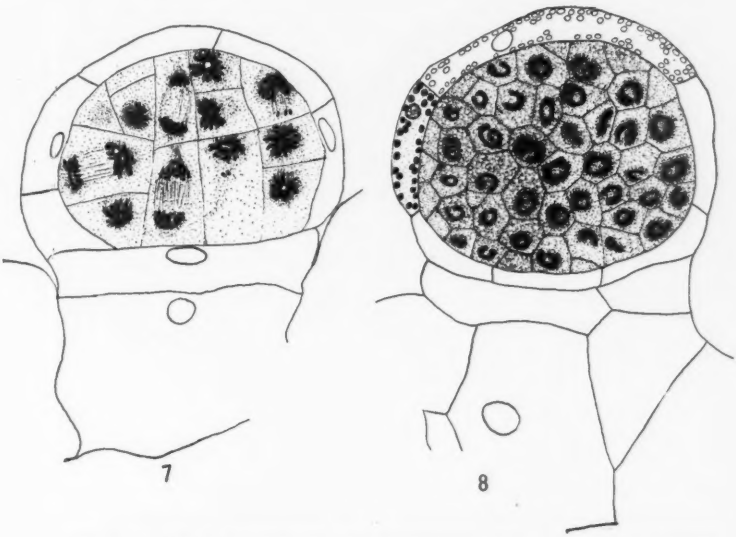
Another type of gametophyte is shown in fig. 6. This prothallium does not branch and is 23 mm. long. There are few or no antheridia on prothallia of this type except near the basal region, but the archegonial cushion is unusually wide and thick, and has a broad row of archegonia along both margins, with more in the center. Transversely, these rows have 1–5 archegonia very closely crowded together. In one gametophyte 16 mm. long, there were found by actual count 142 archegonia on one side of the cushion, with apparently as many on the other side; so that instead of the usual small number of archegonia, there were probably not far from 300 on this particular gametophyte, and other gametophytes of this type. The production of archegonia apparently goes on indefinitely, for the prothallia still looked vigorous.

Antheridia and archegonia

A rather complete series of stages in the development of antheridia and archegonia was examined, but most of them resembled the usual type. Two late antheridial stages are shown in figs. 7 and 8, the former with the spermatogenous cells in mitosis, and the latter with sperms almost mature. There are apparently 16 chromosomes, though none of the figures are in condition to permit absolute accuracy in counting. As a rule, antheridia are found only on the basal region of old prothallia or on the branches, but occasionally one or two appear at any point on the gametophyte. In a few instances immature antheridia were found among mature archegonia. An unexpected feature in archegonial development is their appearance far back from the growing point, so that young archegonia are found among the old ones. A peculiarity several times noted was two archegonia with no wall between the egg cells, but with two complete necks; otherwise nothing unusual was seen in their development.

Fertilization

A few prothallia were placed in water for a short time, and fertilization took place in the usual way, one or more sperms entering the neck and reaching the so-called receptive spot, where one sperm enters the egg. The sperm nucleus fuses slowly with the egg nucleus,



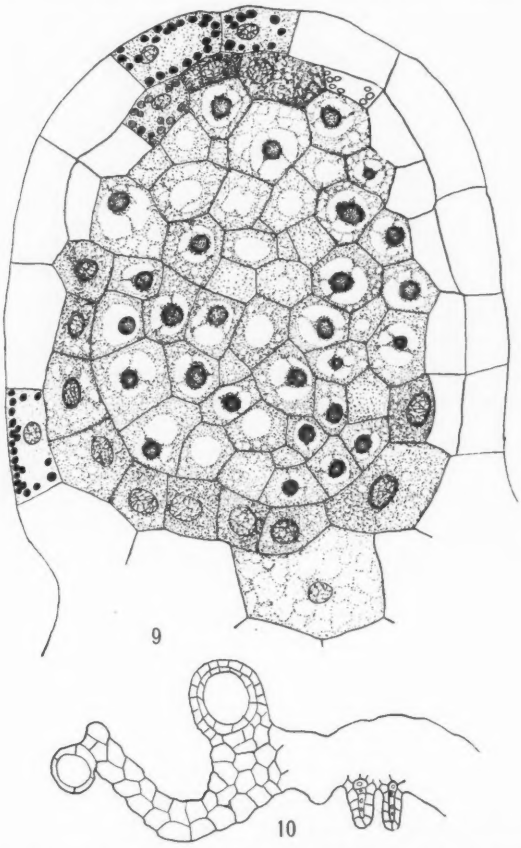
FIGS. 7, 8.—Fig. 7, antheridium with spermatogenous cells in mitosis, showing approximately 16 chromosomes; fig. 8, antheridium with sperms almost mature.

the fusion apparently being completed while both are in the resting condition. A few normal embryos in well-advanced stages were found. These must have been formed from the fertilized egg or have been developed parthenogenetically.

Apogamy

A peculiar structure was found on the gametophyte which bore the antheridia shown in fig. 8, but it was found on the upper surface. The diagram (fig. 10) shows the relation of this body and the antheridium to each other and to the archegonia. It is clear that there are only two possible interpretations of this structure: it must be an

abnormal antheridium, or it is a sporangium; if it is the latter, this is a case of apogamy. The great difference in size is seen by com-



FIGS. 9, 10.—Fig. 9, a sporangium-like structure from the upper surface of the gametophyte from which was drawn fig. 8; two layers of cells outside the fertile region, the inner layer suggesting a tapetum; each layer consists of many cells; fig. 10, diagram showing the relation of figs. 8 and 6 to one another and to the archegonial region.

paring the figures, which are drawn to the same scale. Among all the antheridia examined from this material, there is no such difference in size, the antheridium shown in fig. 8 being a rather large one. In

all the antheridia only one layer of wall cells is present, and these are few and rather definitely placed. But this structure shows two distinct layers (figs. 9, 10), except in one section, and this at only one place (fig. 11). Here for the space of two cells the wall is only one cell thick, but these two cells might divide and make the two layers complete. The outer layer consists of many cells, which always contain chloroplasts, but the next layer contains chloroplasts in a few cells only. The nuclei in this second layer are quite different from those in the interior, and in some of the cells there is a suggestion of the usual tapetal appearance. It does not have the long slender stalk characteristic of the normal sporangia of the higher Polypodiaceae, but it probably would have elongated somewhat before maturity, making it very similar to the stalks of the Osmundaceae. Consequently, this structure is quite unlike an antheridium, and is almost perfect in its sporangial characters. As there were no mitotic figures, it could not be determined whether the nuclei contained the sporophytic or gametophytic number of chromosomes, but we should expect the sporophytic number to appear, especially since another prothallium bore a mature sporangium of the *Osmunda*, type, with a few spores still remaining in the sporangium. In the absence of mitotic figures, such a sporangium easily invites speculation. It

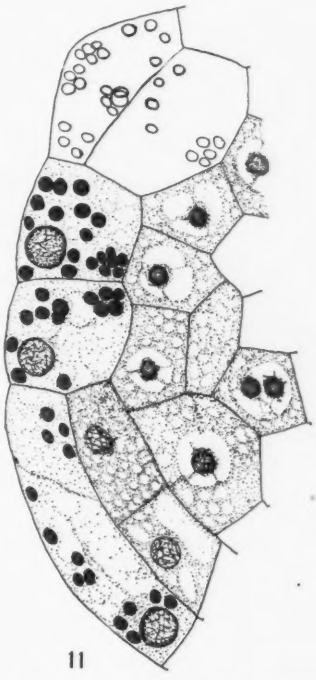


FIG. 11.—A small portion of the same sporangium-like structure shown in fig. 9, but from another section; this is the only place where one layer of cells appears outside the fertile region, and these two cells might divide and make the two layers complete.

may be an x structure, and the spores may have been formed in the usual way, so that the new gametophyte will have only $\frac{1}{2}x$ chromosomes; an interesting surmise in view of the fact that nearly related forms are known to show 12, 24, and 48 chromosomes. Or there may have been a doubling of the number and a subsequent reduction at the formation of spores. The appearance of a sporangium upon a prothallium will be accepted as a case of apogamy.

Discussion

LANG (**2**), in his work on apogamy, grew prothallia from spores. Such prothallia, when kept dry, in direct sunlight, and watered only from below, developed leaves, roots, and ramenta on the prothallia themselves or on the cylindrical processes. The process continued as a leaf, or it produced sporangia, and tracheids were found both in the process and in other parts of the prothallium. When sporangia were found on a cylindrical process, tracheids were always present in the underlying tissue. YAMANOUCHI (**7**) reports very slow growth and few abortive archegonia in his material kept in bright light and dry air.

My material was not in bright light and was given plenty of moisture, being kept in as nearly normal condition as possible, except for the entire absence of liquid water. Consequently, if this is apogamy, the lack of fertilization is apparently the only factor involved in its appearance here, for in this material archegonia were very numerous and were normal in every respect, and fertilization did take place when water was supplied. In this respect it seems to be like *Marsilea*, where SHAW (**3**) and STRASBURGER (**5**) found that if megaspores of *Marsilea Drummondii* were isolated, and therefore fertilization prevented, parthenogenetic (apogamous) embryos were produced.

The gametophyte number of chromosomes is approximately 16. This number is very suggestive of Osmundaceae (SMITH **4**, STRASBURGER **6**, YAMANOUCHI **8**), which is reported to have 16 chromosomes in the spore mother cell. However, the young sporophyte does not resemble the mature form of *Osmunda*, and whether it resembles the sporeling I cannot tell, not being familiar with the sporelings of *Osmunda*.

The sporophyte structures afforded no opportunity for determining whether it contained the haploid or diploid number of chromosomes. YAMANOUCHI found the haploid number of chromosomes in apogamous embryos, and concluded that the number of chromosomes is not the only factor which determines the characters of the sporophyte and gametophyte. STRASBURGER (**5**) found two kinds of megaspores in *Marsilea Drummondii*, some with the haploid and some with the diploid number of chromosomes. It would be expected that those with the diploid number of chromosomes would develop sporophytes without fertilization, as the gametophyte, and consequently the egg, has the diploid number already present. FARMER and DIGBY (**1**) found a vegetative fusion of nuclei in two forms, thus getting the sporophytic number of chromosomes without ordinary fertilization.

The question of apogamy and the literature on the subject will not be discussed further at present, as it is hoped the material may furnish further evidence of this condition.

It is a pleasure to express my obligations to Dr. CHARLES J. CHAMBERLAIN for advice and criticism during this work.

Summary

Prothallia kept for three years in the laboratory in as nearly normal conditions as possible, except for the absence of liquid water, continue to grow, but develop peculiar forms and branching of various types.

The sex organs continue to develop, antheridia being found occasionally on the main plant in all positions, but especially on the branches. Archegonia become very numerous, approximately 300 having been found on one gametophyte. These not only develop in the apical region, but also far back among the old archegonia.

Fertilization may take place whenever liquid water is present, as shown in several cases where gametophytes were placed in water and sectioned later.

Apogamy is present in a sporangium-like structure which lacked the long stalk of the Polypodiaceae, but was not unlike the younger stages of the sporangium of the Osmundaceae. It had two layers of cells outside the fertile region, the inner of these layers resembling a tapetum.

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PERIODICITY IN DICTYOTA AT NAPLES

I. F. LEWIS

(WITH ONE FIGURE)

Periodicity in the production of the sexual cells of *Dictyota dichotoma* has been described by WILLIAMS (**8**) for Bangor, Wales, and Plymouth, and by HOYT (**2**) for Beaufort, North Carolina. At Bangor the sexual products are liberated at fortnightly intervals, the rudiments of sexual organs appearing a few tides before the least neap, and the mature gametes being liberated 3–5 tides after the greatest spring. In October, however, the time relations are reversed. At Plymouth the crops are later, as well as slower in maturing, than at Bangor, liberation occurring 7–12 tides after the greatest spring. At Beaufort only one crop a month is produced, the initiation of rudiments occurring the day before, or the day of, and liberation taking place six days after, the greatest spring tide.

These striking differences in the behavior of what is pronounced to be the same species in different localities make it desirable that careful observations be recorded for this form in other regions than those mentioned, and especially in those regions where the tidal relations are different. WILLIAMS surmises that "the periodicity of the sexual cells is an hereditary character, and consequently may be expected to manifest itself in seas and habitats where there are no tides." OLTMANS (**5**, pp. 487, 488) states: "WILLIAMS findet einen Zuzammenhang der Entwickelung und Befruchtung und mit den bekanntlich in Abständen von 14 Tagen auftretenden 'Spring- und Nipptiden.' Da solche im Mittelmeer fehlen, werden erneute Untersuchungen hier die Dinge zu klären haben."

With these facts in mind, I took occasion, during March and April 1908, to make daily notes on the condition of the sexual plants of *Dictyota* at Naples.[1] The results of these observations are here presented.

[1] I take this opportunity of expressing my thanks to the directors of the Smithsonian Institution for the privilege of occupying a table at the Zoological Station in Naples, and to Dr. R. DOHRN and Dr. LO BIANCO for their cordial cooperation during my stay in Naples.

The range of the tides at Naples is very much less than at either Beaufort or Bangor, though it cannot be said that tides are wholly lacking at this point. The daily readings of the tide gauge near Naples, furnished by the Italian government, agree closely with the tides predicted by the U.S. Coast and Geodetic Survey. Extraordinary conditions of wind and weather, however, may affect the range of the tides very appreciably. For the period studied, the maximum daily range of the tides was 2.1 feet, the minimum 0.4, the average 1.0. The water level varied from 0.7 feet below to 1.5 feet above mean low water of spring tides. The following table gives an idea of the relative ranges of the tides at Bangor, Beaufort, and Naples.

TABLE I

	Average range	Difference in height of low water at spring and neap tides
Bangor	17.9 feet	5.7 feet
Beaufort	2.8	0.5
Naples	1.0	0.25

The sexual cells of *Dictyota* were found to be produced at regular intervals, the time of initiation of the rudiments and liberation of the mature gametes bearing a definite relation to the periodic changes in the tides. The crops are borne, as at Bangor, at fortnightly intervals. Initiation of the rudiments occurs on the same day as general liberation of the mature gametes, this being two or three days after the least neap tide. The number of days required for the development of a single crop is approximately sixteen. The development of the sori is fairly uniform, not being accelerated at the time of the spring tides.

A comparison of the facts of periodicity in *Dictyota* at Bangor, Beaufort, and Naples is given in the following tables.

The accompanying chart (fig. 1) gives in graphical form the record of one crop from initiation to liberation, showing the relations between the tidal changes and the fructification of *Dictyota*. The chart should be compared with the charts given by HOYT (**2**, pp. 386, 387) in order to compare the behavior of *Dictyota* at various localities.

At any given time all the individual plants, collected at various points from Capo Miseno to Santa Lucia, are at approximately the same stage of development. There are noticeable differences, however, as for instance in the plants collected on April 10. According to the chart, this date shows the beginning of liberation of gametes,

TABLE II

	Initiation	Liberation	Time of development
Bangor	A few days before the the least neap	3–5 tides after greatest spring	12–17 days
Beaufort.....	Day before or day of greatest spring	6 days after greatest spring	8–9 days
Naples.....	2–3 days after least neap	2–3 days after least neap	16 days

TABLE III

HISTORY OF A SINGLE CROP

Day	Beaufort	Bangor	Naples
1.....	Undivided rudiments	Undivided rudiments	Undivided rudiments
3.....	8–16 cells in surface view of antheridia	Undivided rudiments	Undivided rudiments or 2–4 cells
4.....	32–64 cells	Undivided rudiments	2–4 cells
5.....	64 cells	Undivided rudiments	2–4 cells
6.....	General liberation		2–4 cells
7.....	Belated sori	2–4 cells	4 cells
8.....			4–8 cells
9.....			8–16 cells
10.....		Many sori. 64 cells	16 cells
11.....			16–32 cells
12.....		General liberation	32–64 cells
13.....		Belated sori	64 cells
15.....			General liberation
16.....			Belated sori

and also the beginning of initiation of rudiments. As a matter of fact, the numerous individuals examined on this day showed considerable differences among themselves. The male plants may be grouped in the following categories: (1) no antheridia empty, new crop not visible; (2) none empty, new crop inconspicuous; (3) very few antheridia empty, new crop conspicuous; (4) antheridia empty near base of plant, not empty near apex, new crop barely visible; (5) antheridia as in no. 4, new crop conspicuous, some of the rudiments 4-celled in surface view; (6) antheridia nearly all empty, new crop

barely visible; (7) antheridia all empty, new crop showing 4 cells. The majority of plants collected on this day were in the fourth or fifth stage.

The tendency toward periodicity in the production of sexual cells is probably a hereditary character. That periodicity is not a series of simple responses to successive stimuli is shown by the observation of WILLIAMS that individuals removed from the influence of the tides continue to show the usual periodicity, and by HOYT'S statement that individuals produced by vegetative multiplication

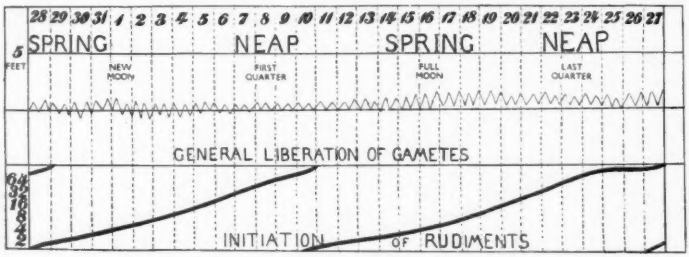

FIG. 1.—Chart showing tidal relations at Naples from March 28 to April 27, 1908, with record of crops of *Dictyota* for the same period. The tide records were furnished by the Italian government, through the kindness of Dr. R. DOHRN, of the Zoological Station at Naples. The chart is to be compared with similar charts for Bangor, Wales, and Beaufort, N.C. (HOYT **2**). The zigzag lines show the rise and fall of the tide and the height of the water in relation to the mean low-water mark of spring tides. The curved lines show the development of the sexual crops (male) of *Dictyota* for the respective periods with their relations to the tides. The numbers opposite the crop curves indicate the number of cells seen in the surface view of each antheridium.

and never subjected to the influence of the tides show approximately the same periodicity as those in their natural habitat. The former observation was confirmed by me at Naples, and goes to show that the habit of periodical reproduction is fairly well fixed. The habit, however, must have had its origin in response to external conditions, and it is a matter of considerable interest to ascertain, if possible, what factor or factors gave rise to the periodicity. The only suggestion made thus far is that of WILLIAMS that the effective factor is the increased illumination during low water of spring tides, a view from which HOYT dissents.

WILLIAMS' hypothesis seems inadequate to explain the periodicity at Naples. *Dictyota* flourishes there at a depth of many feet below

the surface, and the difference between the height of low water at spring and neap tides is only 0.25 feet. This slight difference can hardly cause any considerable variation in the total illumination of the plants under consideration, not as much as is caused by alternating cloudy and sunny days. It is interesting to note, however, that both initiation and liberation occur at Naples on the day that low water occurs at or nearest midday. Thus low water at midday occurred at Naples on March 29, April 10, and April 27, 1908, the days when initiation and liberation were found to occur. Whether this is more than a coincidence is still to be seen, but the fact remains that the critical points in the sexual life of *Dictyota* coincide exactly with the periods of maximum intensity of illumination.

If one considers the behavior of *Dictyota* at Naples alone, it seems a fairly satisfactory hypothesis that the effective factor in producing periodicity is the stimulus of the maximum intensity of light. When one comes to apply this hypothesis to the other regions where *Dictyota* has been studied from this standpoint, it becomes evident that, if true at all, the hypothesis is modified by other factors. At Beaufort, for instance, low water at midday occurs two days before new and full moon, while initiation occurs on the day before or the day of the greatest spring tide. At Bangor, on the other hand, low water at midday occurs about five days before new or full moon, and initiation takes place one to two days before the least neaps. At Plymouth low water of the greatest spring tides occurs at midday, and here the times of initiation and liberation coincide more nearly with the periods of neap tides. It is obvious, then, that the simple explanation that might suffice for Naples is not sufficient for other localities, and that the operative factor or factors must be sought by further investigation.

It seems possible that *Dictyota*, in adapting itself to differing conditions at various localities, has acquired its habit of periodicity in response to different factors. That a similar stage in the reproduction of other algae may be induced by different stimuli has been shown by FREUND (1) to be the case in *Oedogonium* and *Haematococcus*. In these forms the external conditions leading to the formation of zoospores differ according to the condition of the individual plants. For instance, cysts of *Haematococcus* form zoospores

(1) when transferred from foul to distilled water, (2) when brought from darkness into light, (3) when water is replaced by sugar solution. KLEBS's investigations (**3**) illustrate the same point for other algae and fungi. Since the fructification of other forms may be induced by different factors, it is possible that the same may be true for *Dictyota* in various situations, and that the factors concerned in inducing periodicity may vary with the locality.

Other organisms, both animals and plants, show a periodicity in the release of sexual cells comparable to that of *Dictyota*. In *Amphitrite ornata*, an annelid worm, "practically all sexual products are deposited within three days of spring tides" (SCOTT **6**, p. 332). KUCKUCK finds (**4**) in *Nemoderma tingitana* that the gametes are released at times of neap tides. TAHARA shows (**7**) that the oospheres of *Sargassum enerve* are released at fortnightly intervals at about the time of new and full moon. Other species of *Sargassum* are said by TAHARA to show a similar periodicity, in which liberation occurs at various intervals after the greatest spring tides.

It is obvious, therefore, that periodicity in the release of sexual cells is a widespread phenomenon, probably to be attributed to various factors in different species, and perhaps to more than one factor in the same species in different localities.

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CURRENT LITERATURE

BOOK REVIEWS

Fungous diseases of plants

Owing to the existence of an extensive system of experiment stations in the United States, one of whose chief activities has been the investigation of plant diseases, the conditions for the accumulation of facts relating to plant pathology have been unusually favorable. Following the progress made in investigation, the teaching of plant pathology has begun to develop chiefly in the agricultural colleges associated with the stations. Thus far, however, there has been no attempt to organize into a comprehensive text the vast material accumulated by plant pathologists and to make it available for teachers, although a need for such work has been felt by those who have attempted to teach the subject. The appearance of DUGGAR's book[1] on plant diseases is therefore both timely and desirable. The work, as the preface indicates, is designed primarily as a textbook, but its possible service as a reference book has also been kept in view. Aside from the brief historical introduction, it falls into three parts: (1) culture methods and technic, (2) physiological relations, and (3) fungous diseases of plants.

The first part is designed to introduce the student to the methods and manipulations used in the study of fungous diseases. In it are treated the methods of handling apparatus, the preparation of culture media, the cultivation of organisms, and microscopical technic. On the whole, the directions are clear and to the point, and embody many details of manipulation which are acquired only through intimate experience with such work. The use of the freezing method for cutting sections should perhaps have been mentioned, especially as its adaptability for certain kinds of work has been recently emphasized.[2]

The part on physiological relations comprises a discussion of the germination of spores and the modes of life and relation to environmental factors of parasitic fungi, together with chapters on artificial infection, disease control, and the preparation of fungicides. It stands for the whole field which belongs peculiarly to the general subject of plant pathology. Considered from this standpoint, the treatment is surprisingly brief and much that is in the chapter does not belong there. This is especially true of that part of the chapter on germination which deals with methods, part of the section on environmental factors, and nearly all of the chapter on artificial infection. These should have been included in the

[1] DUGGAR, B. M., Fungous diseases of plants. 8vo. pp. xii + 508. *figs. 240.* Boston: Ginn & Co. (undated).

[2] FREEMAN, E. M., The ether freezing microtome in botanical work. Science N.S. **25**:747–749. 1907.

first part on methods and technic. The field represented by this part has been so greatly enriched in recent times, by the addition of both facts and ideas, that the treatment seems wholly inadequate.

The last part, making up the bulk of the work, deals individually with the plant diseases induced by fungi, under which the author includes the myxomycetes and bacteria. The arrangement is in the order of taxonomic sequence, each chapter representing one of the large divisions of the fungi, as Phycomycetes, Ascomycetes, etc. The individual diseases are treated in numbered sections of the chapters. A section is given to the discussion of each disease, except those of minor importance, which are grouped together. The arrangement serves to bring out the morphological relations of the disease-producing fungi, without laying too much stress on purely morphological and taxonomic features. Some clearness would have been gained if the discussion of orders and families had not been forced into the system under headings coordinate with those under which the individual diseases are discussed. The treatment of the diseases is clear and comprehensive, each being discussed with reference to its distribution, the influence of environmental factors on its occurrence and prevalence, the life history of its causal organism, and the methods of its control. In the relative prominence given to the various diseases, the author has been guided by their economic importance, but the scope has been made broad enough to include all of the common diseases injurious to cultivated crops. A few diseases not occurring in this country have been included, apparently for the sake of completeness. As a rule, the text is conservative and free from innovations at variance with current usage. There is one notable exception, however, in the introduction of a series of newly compounded terms to apply to certain artificial groups of the rusts, based on the number of spore-forms present in the cycle. Thus we have "euautouredo" to include all autoecious rusts possessing all spore-forms, and "opsiautouredo" to include all autoecious rusts lacking the uredo stage, etc. Aside from any criticism that may be offered on account of the faulty composition of these unwieldy terms, the pedagogical soundness of introducing them for the first time through the medium of a textbook may well be questioned.

In matters of detail, the work shows an unusual lack of care in the preparation of the manuscript or in proofreading. The following examples serve as illustrations: on page 54 "Löwitz" is printed for "Löwit"; on page 86 "Scot" for "Scott"; and on page 121, second citation, "Histology" for "History." In the legend of fig. 50 a germinating oospore is described as a "germinating oogonium." On pages 272 and 276 "VON SCHRENK" should read "VON SCHRENK and SPAULDING." On page 337 the citation of MCALPINE should read "Stone-fruit trees" instead of "Stone fruits." *Phoma Betae* is said not to occur in the United States (p. 344), but it has been reported from Colorado and Kansas.[3] On page 386, note, ARTHUR is said to have introduced the terms "*pycnium, aecium,*

[3] ORTON, W. A., Plant diseases in 1907. Yearbook Dept. Agr. 1907:577–589; see also Yearbook 1906:502 (*Phyllosticta Betae* Oud.).

uredinium, and *telium* in substitution for teleuto, uredo, aecidial, and spermagonial stages" of the rusts, instead of the reverse order. On page 467 *Trametes Pini* is said to be the "chief cause of loss among fungi."

On the whole, the book is an excellent presentation of the subject of plant pathology from an American standpoint. Most of its shortcomings relate to individual or minor details. In it the vast amount of material collected through the agencies of the experiment stations and the U.S. Department of Agriculture has been brought together for the first time in an easily available form. The facts presented are largely derived from American work and apply to American conditions. It is sufficiently comprehensive for a textbook, and will be of much service as a reference book in the field which it represents. The style is clear and concise, and the arrangement is that which the teacher would naturally adopt. The free citation of literature is of great service to both student and teacher. The book is abundantly illustrated, and both illustrations and press work are all that could be desired.—H. HASSELBRING.

The morphology of plants

The third and last volume of VELENOVSKÝ'S textbook[4] on the comparative morphology of plants deals with the flower of phanerogams, the ovule, pollination, embryo, seed, fruit, and the evolution of plants. Fertilization, parthenogenesis, and polyembryony are treated under the section on the ovule, preceding the description of pollination. The volume opens with the following definition of a flower: "The flower of phanerogams is a shortened axis of limited growth, which carries foliar organs adapted to the purposes of fertilization." We are assured that this definition applies to all cases except the female structures of the genus *Cycas*, which are not regarded as flowers.

The book deals almost entirely with the grosser external features of plants, little attention being given to the details of development. It must be confessed that the phase of morphology represented by this book is somewhat neglected by modern morphologists, who are likely to pay insufficient attention to the taxonomic side of botany. Morphologists should find the work useful as a reference and as a supplement to their taxonomy; but as a complete textbook of morphology it is not comprehensive enough to meet modern demands.—CHARLES J. CHAMBERLAIN.

NOTES FOR STUDENTS

The cretaceous plants of Japan.[5]—This interesting product of the Anglo-Japanese understanding represents the structural study of partly calcified and partly silicified nodules from the Upper Cretaceous of Hokkaido in northern

[4] VELENOVSKÝ, JOS., Vergleichende Morphologie der Pflanzen. Vol. III. pp. 478. *pls. 6–9. figs. 400.* Prag: Fr. Řivnáč. 1910. For review of vols. I and II see BOT. GAZETTE **44**:310. 1907.

[5] STOPES, MARIE C., and FUJII, K., Studies on the structure and affinities of cretaceous plants. Phil. Trans. Roy. Soc. London B **201**:1–90. *pls. 1–9.* 1910.

Japan. Only in rare instances were the authors able to make out the external form of the material studied, and in no case do they seem to have been able to correlate it with the extremely abundant cretaceous genera known from impressions. To this initial disadvantage is added a not entirely satisfactory familiarity with the anatomical structure of living angiosperms and conifers. The eighteen species described as new in the memoir are consequently in some cases not really new, since they represent the parts of plants already known from impressions and recently identified structurally by American paleobotanists. In other instances the anatomical characterization is too vague and indefinite for subsequent use. In spite of these drawbacks, the memoir under discussion must rank as one of the most important recent contributions on the cretaceous flora, and it is much to be desired that the authors may be able to continue their investigations as they promise to do.

Of the eighteen species described, four are cryptogamic, one being a fungus and three others ferns. An interesting cycad-like leaf, *Niponophyllum*, is described which differs from the leaf structure of living cycads in the complete absence of centrifugal wood, all the xylem being of the cryptogamic centripetal type.

Of the other gymnosperms described, the most interesting is *Yezonia*, which is considered by the authors to represent a new genus, and of which they state "it is impossible to find any family among the gymnosperms with which we can satisfactorily include this plant." This view of the matter will hardly stand, since in every detail of structure it corresponds absolutely with *Brachyphyllum*, the commonest conifer of the later Mesozoic, which, moreover, on anatomical grounds has been recognized recently as an araucarian conifer. Another gymnospermous branch is also described under the new generic name *Cryptomeriopsis*. Of this it may be stated that the description given of its internal organization by the Anglo-Japanese authors parallels with fidelity, so far as it goes, that of *Geinitzia Reichenbachi*, recently described structurally from the North American Cretaceous. Two imperfect coniferous cones are likewise characterized, *Yezostrobus* and *Cunninghamiostrobus*. One *Araucarioxylon* and two species of *Cedroxylon* complete the list of coniferous remains.

Either as the result of a bad condition of preservation, or a failure to realize clearly the importance of detailed description, four angiospermous ligneous genera, all considered to be new (*Jugloxylon*, *Populocaulis*, *Fagoxylon*, *Sabiocaulis*), are insufficiently characterized. The detailed structure of the rays, the characters of the vessels and wood fibers, as well as the distribution of wood parenchyma, all important features in the description of angiospermous fossil woods, are entirely or almost entirely omitted. If the omission is due to faulty preservation, the woods are scarcely worth publishing. The genus *Saururopsis* is somewhat more clearly characterized. One genus (*Cretovarium*) representing a tricarpellary ovary is likewise described, but as the accompanying vegetative organs and even any considerable part of the floral apparatus itself are absent, it seems impossible to arrive at any satisfactory conclusion as to its affinities. One curious and unfortunate omission throughout the memoir is the almost com-

plete failure to indicate the magnification used in the figures. This makes comparisons on the part of other workers difficult or even impossible.

In spite of the exceptions taken in various respects to the work of the Anglo-Japanese authors, it must be conceded that their line of investigation is one of great promise, and it is to be hoped that they will feel encouraged to continue it with a greater attention to definiteness in anatomical characterization.—E. C. JEFFREY.

Vascular anatomy of Gleichenia.—BOODLE and HILEY,[6] from the study of the anatomy of *Gleichenia pectinata* and allied species, reach certain theoretical conclusions as to the origin of the tubular medullated stele. They report the result of the examination of the node and internode, as well as the branching stem, of certain species of *Gleichenia*, particularly *G. pectinata*. It is not surprising that they reach substantially the conclusions which have been published already by the senior author in earlier contributions. The published results in this case, however, appear to indicate a certain modification of the position originally held by BOODLE, to the effect that in all cases the pith is a part of the stele and is not derived by inclusion of the fundamental or ground tissue from outside the central cylinder; for the authors in this article use the term solenostelic, borrowed from GWYNNE-VAUGHAN and employed by him in the sense of a tubular stele with internal as well as external phloem and inclosing fundamental tissue as a pith (a meaning stated by GWYNNE-VAUGHAN himself to be equivalent to the reviewer's siphonostelic with internal phloem). Although the English writers in this instance concede apparently the arrival of the solenostelic condition as the final result of the modification of the pithless protostele, they express the opinion that the pith appears first as the result of the transformation of some of the tracheids into a central mass of parenchyma, a condition followed by the appearance of ramular gaps in the stele as the result of branching, leading to the intrusion of phloem from the outside of the stele and ultimately of the fundamental tissue itself. Only at the end of the process do the leaf gaps appear and become patent.

These views are all the more remarkable because in the same article the authors concede that the islands of parenchyma occurring in the petiolar strands of certain representatives of the Gleicheniaceae are derived from the cortex by inclusion, and were originally surrounded both by internal phloem and internal endodermis. The condition in which the included parenchyma is separated from the vascular tissues of the petiole by neither endodermis nor internal phloem is a result of progressive degeneracy. It appears almost an extreme example of the perversity of the human mind to explain the occurrence of central parenchyma in the leaf trace in a node diametrically opposite to that adopted for the appearance of a pith in the vascular tissues of the stem. If the fundamental tissues may be included in the leaf trace, there appears to be no reason why a similar process should not lead to the formation of pith in the axis. The adoption of this hypoth-

[6] BOODLE and HILEY, On the vascular structure of some species of *Gleichenia*. Annals of Botany **23**:419–432. *pl. 29*. 1909.

esis, moreover, explains the appearance of included endodermis and phloem in young individuals of various examples of vascular plants, a condition which finds no elucidation whatever in connection with the views adopted almost universally by English anatomists.—E. C. JEFFREY.

Jurassic woods.—GOTHAN[7] describes a number of fossil woods from the Jurassic of King Karl's Land, none of which he refers to the Araucarineae. Two species, *Phyllocladoxylon* sp. and *Xenoxylon phyllocladoides*, are considered to belong in the region of the Podocarpineae. One *Cupressinoxylon* is also figured, which is compared with the *C. McGeei* of KNOWLTON. Next are two Cedroxyla, *C. cedroides* and *C. transiens*. To the latter of these he attaches much importance because of the presence of araucarian pitting together with the ray structure of the Abietineae. The author assumes that he has to do in this instance with an abietineous wood, in which certain araucarineous characters indicate the derivation of the Abietineae from an araucarian stock, in accordance with the conventional and generally received views. It does not seem to have occurred to the author that he may have an araucarian conifer with indications of transition toward the Abietineae. The genus *Araucariopitys*, recently described by the reviewer, has the same ligneous characters as GOTHAN's *Cedroxylon transiens*, only with a more pronounced resemblance to the Abietineae. It is nevertheless unquestionably an araucarian conifer. One abietineous wood is described, namely *Protopiceoxylon extinctum*. The wood in question has normally only horizontal resin canals, but vertical ones may occur as the result of injury. The author regards his wood as evidence that the vertical type of resin canal is older than the horizontal, forgetful that GOEPPERT and PENHALLOW have described much older Pityoxyla from the Carboniferous and Permian respectively, which show only horizontal canals. It seems, accordingly, that "*Protopiceoxylon*," with a much greater degree of probability, represents a stage in degeneracy toward the *Cedroxylon* type, rather than a primitive abietineous type antedating *Pityoxylon*. The author lightly sets aside the evidence adduced on comparative anatomical and experimental grounds by the reviewer for the great age of the pinelike Abietineae. With admirable Teutonic frankness, he characterizes the reduction hypothesis of the phylogeny of the Coniferales as one to which, "wohl niemand beipflichten kann." He apparently loses sight of the fact that the reduction hypothesis is now quite generally adopted by competent morphologists for such degenerate groups as the Equisetales, Lycopodiales, etc. Had the literature on the fossil conifers, which has recently been published by American paleobotanical writers, been available, in all probability GOTHAN's opinion in regard to the fallacy of the reduction hypothesis in connection with the phylogenetic development of the conifers would have been expressed with considerably more reserve.—E. C. JEFFREY.

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[7] GOTHAN, W., Die Fossilen Hoelzer Koenig Karls Land. Handl. Kgl. Svensk. Vetensk.-Akad. **42**: no. 10.

Chemotropism of roots.—In a preliminary paper, PORODKO[8] reports upon the chemotropism of the roots of *Lupinus albus* and *Helianthus annuus*. Roots 20–35 mm. long were placed in a lamella of agar varying in thickness from 6 to 60 mm., which separated the solution used from water. In all, 50 chemical substances were used, the concentration of which varied from 0.1 *n* to 0.001 *n*. As a rule, the roots did not remain straight, but bent against or with the diffusion stream. The range of concentration between maximum and minimum depended upon the substance used and the thickness of the agar lamella. Concentrations close to the maximum caused bending against the diffusion stream or positive response, which effect was observed with both electrolytes and non-electrolytes. PORODKO considers this a traumatropic response, due to the inhibition of growth on the up-stream side of the root. With lower concentrations, electrolytes and non-electrolytes affect the roots differently. The former cause great regularity as regards the direction of bending of the root, while the latter produce positive, negative, and intermediate responses. Acids, alkalies, and carbonates cause positive, and neutral salts negative bendings. The responses due to H and OH ions are considered to be traumatropic. The amount of negative response seems to depend upon the cation, being greater in the presence of one with a double charge than in the presence of one with a single charge. In many cases the responses are not all of one kind. Nevertheless, it is necessary to explain the cause of all. From his experiments, PORODKO concludes that positive but not negative responses can take place in decapitated roots, and that the latter, but not the former, show up as after-effects, although only on the clinostat. The two reactions are different in nature, the positive being passive and caused by the inhibitory effect of the greater concentration on the growing region on the up-stream side of the root, the negative being active and due to the chemotropic effect of the diffusion stream, which tends to accelerate the growth on the up-stream side. Hence, upon the growing region of a root of *Lupinus albus* subjected to the influence of the diffusion stream of a chemical substance, two antagonistic tendencies are at work, the direction of bending of the root being dependent upon the relative strengths of the two tendencies. Roots of *Helianthus annuus* act differently from those of *Lupinus albus*, in that they show only traumatropic response, but why this is true is not known.—R. CATLIN ROSE.

National Academy of Sciences.—At the annual session of 1910 two botanical papers were presented (April 19), which may be outlined as follows:

"The distribution of *Agave* in the West Indies," by WILLIAM TRELEASE.—Three main types of *Agave* are recognized in the West Indies: one confined to the southwestern Cuban region, another to the Inaguas, and the third ranging through the entire archipelago. Subtypes of the latter are limited respectively to the Greater Antilles, the Bahamas, the Caribbees and the Leeward Islands, and the

[8] PORODKO, THEODOR, Ueber den Chemotropismus der Wurzel. Ber. Deutsch. Bot. Gesell. **28**:50–57. 1910.

adjoining Venezuelan coast. Within these groups specific differentiation is observable, so that each island isolated by a 100-fathom channel has its endemic species, the islands with a common coastal plain possessing little if at all differentiated forms. The almost entire absence of the genus from South America and the geographic grouping of species and superspecies in the West Indies indicate that *Agave* penetrated from the Central American mainland, where it centers, and overran the terrain before the disruption into islands, two or perhaps three parent stocks being involved.

"The vascular plate and cotyledons of gymnosperms," by JOHN M. COULTER.—Among the various vascular structures of gymnosperms that have been used to suggest progressive evolutionary changes, the vascular plate of the cotyledonary node is perhaps as significant as any, especially its connections with the cotyledons. Series of cycads, of conifers, and of other gymnosperms were shown to illustrate the following general tendencies: to reduce the cotyledons to two, to reduce the protoxylem poles of the vascular plate (and hence the root poles) to two, to eliminate certain vascular connections of the cotyledons, and to restrict the branching of strands within the cotyledons.

Differentiation among chromosomes.—*Crepis virens* seems to afford promising material for the solution of several difficult cytological problems. JUEL had already found the diploid and haploid numbers in *C. tectorum* to be 8 and 4. ROSENBERG[9] now finds the numbers in *C. virens* to be 6 and 3, the lowest numbers yet established for plants. The fact that the chromosomes are so few and that they are readily recognized as "prochromosomes" in the resting nucleus, removes any danger of uncertainty in counting which might be anticipated in case of large numbers. Not only is the number low, but the individual chromosomes are not alike, two being long, two rather short, and two intermediate. In the diploid divisions these three kinds of chromosomes appear in pairs, the members of a given pair being alike. At synapsis a double thread appears, and there is a fusion which is to be regarded as a fusion of whole chromosomes, reduction in number being brought about in this way. ROSENBERG suggests that interesting results might be obtained by crossing *Crepis virens* and *C. tectorum*, and he promises to make the attempt.

Noting the low number of chromosomes in *Crepis tectorum* (8 and 4) and in *C. virens* (6 and 3), TAHARA[10] examined the Japanese species, *C. japonica*, and found the numbers to be 16 and 8, just double the numbers in *C. tectorum*. The chromosomes were also found to be of different sizes and forms. If there is a relation between specific characters and chromosomes, the genus *Crepis* would seem to the reviewer to be a favorable form for the investigation of this subject.—CHARLES J. CHAMBERLAIN.

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[9] ROSENBERG, O., Zur Kenntniss von den Tetradenteilung der Compositen. Svensk. Bot. Tidskrift **3**:64–77. *pl. 1.* 1909.

[10] TAHARA, M., Ueber die Zahl der Chromosomen von *Crepis japonica* Benth. Bot. Mag. **24**:23–28. *pl. 2.* 1910.

Interaction between scion and stock.—MEYER and SCHMIDT[11] have produced a voluminous article on the interchange of substances and mutual influence between stock and scion in a heteroplastic graft. The introduction and review of literature occupy 48 pages, and 33 pages are given to the statement of results and the summary. One is compelled to think that the article could have been advantageously condensed to half the space. The authors mention that interchange of carbohydrates was already fully worked out, while the previous work on movement of aplastic and other substances is very unsatisfactory. They direct their attention to the movement, formation, and storage of alkaloids, using *Nicotiana Tabacum* as scion on *N. affinis* and *Solanum tuberosum* as stocks, and *Datura Stramonium* as scion on *Solanum Lycopersicum* and *S. tuberosum*. They find that alkaloids can pass from scion to stock; but the movement is very slow, and apparently takes place through the parenchyma and not through the sieve tubes. With *N. Tabacum*, normally rich in nicotin, as scion, and *N. affinis*, normally poor in nicotin, as stock, the latter comes to contain many times its normal amount of nicotin, and even ten times as much as the scion; while the scion becomes relatively poor in it. With *S. tuberosum* as stock for *N. Tabacum*, the periderm cells of the former become the main storage tissue for the nicotin. It is most abundant in the tissue of the stock just below the graft, and decreases in amount as the cells are more distant; while in the tuber none at all or only traces appear.—WILLIAM CROCKER.

Living cells and extreme temperatures.—GEORGEVITCH,[12] in investigating the effect of extreme temperatures on living cells, used the root tips of *Galtonia candicans*. They were kept at 40° C., and −5° C., killed and fixed at the same temperature, and the effect of these extremes noted. At a temperature of +40° C., the cytoplasm coagulates and forms small clumps of dark-staining material. At low temperatures the cytoplasm becomes vacuolate, and the coordination in the action of the spindle fibers is broken up, which results in the distribution of chromosomes between the poles. The activity of the kinoplasm is decreased by low temperature and increased by high temperature. There result larger spindles with stronger fibers, more rapid transport of chromosomes, and shortened duration of nuclear division. Thereby cell wall formation is inhibited and binucleate cells are of frequent occurrence. The chromosomes often form chains, due to the irregular transport toward the poles. In cold preparations the nucleus often takes on an amoeboid form, also in the warm, but not to such an extent. In general it can be said that high temperatures favor development of chromatic

[11] MEYER, ARTHUR, and SCHMIDT, ERNST, Ueber die gegenseitige Beeinflussung der Symbionten heteroplastischer Transplantationen mit besonderer Berücksichtigung der Wanderung der Alkaloide durch die Pfropfstellen. Flora **100**:317–396. *figs.* 3. 1910.

[12] GEORGEVITCH, PETER, Ueber den Einfluss von extremen Temperaturen auf die Zellen der Wurzelspitze von *Galtonia candicans*. Beih. Bot. Centralbl. **25**:127–135. 1910.

material, while low temperatures inhibit it. In cold preparations one finds collections of chromatin which stain blue and are called pseudonucleoli. SCHRAUMEN found the same in the cells of shoots of *Vicia Faba* kept at both high and low temperatures. GEORGEVITCH did not find them in warm preparations. In cold preparations the nucleoli show an increase in size, mass, and numbers.—R. CATLIN ROSE.

Fossil Osmundaceae.—KIDSTON and GWYNNE-VAUGHAN[13] have continued their interesting investigations on the fossil Osmundaceae. In the case of the most important of the species which they describe (*Thamnopteris Schlechtendalii* Eichwald) there can apparently be no doubt that they have really to do with the remains of an osmundaceous fern. They find that in this species the center of the stele is marked by the presence of a mass of short tracheids without any admixture of parenchyma, which curiously enough they regard as the equivalent of a pith. It is surely begging the question as to the origin of medullary structures, to regard tissues which admittedly are entirely tracheary and contain not the slightest admixture of parenchymatous cells as equivalent to the medulla of the higher plants. The difficulty of regarding the central mass of short tracheids in *Thamnopteris* as a pith is rendered insuperable, apparently, by the fact that the leaf traces originate from the stele exactly as in those cases where no pith is present, that is without giving rise to any foliar gaps. The views entertained by the present authors and the majority of English writers on anatomy encounter an additional difficulty in that they are quite unable on their hypothesis to explain the presence of internal phloem and internal endodermis in closed steles. These find apparently a very simple and natural elucidation in connection with the reduction theory now advocated by a considerable number of American anatomists.—E. C. JEFFREY.

Bennettitales.—NATHORST[14] has described the more or less complete reproductive apparatus of a number of bennettitean forms. There are three species of *Williamsonia* from the Jurassic beds of Whitby and Scarborough, England. In these were found in different cases both microsporangia with microspores, and seeds. The structure of the microspores is illustrated by admirable photomicrographs. A new genus (*Wielandiella*) has a very remarkable vegetative organization. The stem branches freely in an apparently dichotomous manner and is quite slender. The cones occur in the forkings of the branches. The vegetative structure resembles that of the problematic *Anomozamites*. The cones showed remains of both pollen and seeds. The structure of the microspores of a third genus (*Cycadocephalus Sewardi*) is described. These are remarkable for their close resemblance to fern spores. For comparison, a figure of *Wel-*

[13] KIDSTON, R., and GWYNNE-VAUGHAN, D. T., On the fossil Osmundaceae. III. Trans. Roy. Soc. Edinburgh **46**:1909.

[14] NATHORST, A. G., Paleobotanische Mitteilungen. 8. Handl. Kgl. Svensk. Vetensk.-Akad. **45**: no. 4. 1910.

trichia Fr. Braun from the Mesozoic of Franconia is introduced. The result of the present important communication is to enlarge our knowledge of the male organs of the Bennettitales by seven different species belonging to five different types. Two species of *Williamsonia* have monosporangiate strobili. The same condition is clearly demonstrated in *Cycadocephalus*. The author wisely refuses to commit himself as to the nature of the Bennettitean inflorescence, and avoids any reference to its possible homology with the angiospermous flower. —E. C. JEFFREY.

Rôle of ammonium salts.—PRIANISCHNIKOW,[15] working with grasses, has already shown that a substitution in sand cultures of $\frac{1}{4}$–$\frac{3}{4}$ of the $NaNO_3$ by $(NH_4)_2SO_4$ increases the power of the plant to gain phosphoric acid from raw phosphates (phosphorite), while in absence of $(NH_4)_2SO_4$ the plants show phosphoric acid starvation. Total substitution, however, greatly reduces the harvest. Both these effects are attributed to the released sulfuric acid. In partial substitution the acid was strong enough to aid in dissolving the phosphorite, and in total substitution so strong that it greatly injured the plants. It is also shown that $CaCO_3$ is very effective in preventing injuries by $(NH_4)_2SO_4$, and if only $\frac{1}{4}$–$\frac{1}{2}$ enough was used to neutralize the liberated sulfuric acid, the consumption of the phosphorite was also much favored. In working with barley, peas, and buckwheat, the author has determined that mixtures of $NaNO_3$ and $(NH_4)_2SO_4$ are better sources of nitrogen than either one alone, for, as he states, the first is physiologically basic (base liberated due to the consumption of NO_3 as source of nitrogen) and the second physiologically acid (acid liberated due to the consumption of NH_4 as the source of nitrogen). The two maintain the culture medium neutral. The author does not attempt to decide between the relative values of ammonium salts and nitrates as a source of nitrogen when the former are of very weak acids, as those used by RITTER[16] to settle this question for fungi.—WILLIAM CROCKER.

Fossil conifers.—NATHORST[17] has described with truly admirable clearness and judgment the cones of the problematical coniferous genus *Palissya* from the Rhaetic of Schonen in Sweden. The ovuliferous cone scales are characterized by the presence of two rows of opposite seeds, with very loose integuments or epimatia. The author concludes that the evidence of the organization of the cone scales tends to connect the genus with a second genus described in the article, namely *Stachytaxus*. This genus has yewlike foliage, and attached to the ends of the twigs are lax cones with distant scales, each of which bears two ovules, provided with a widely flaring integument or possibly an epimatium comparable with that found in the Taxineae. The author argues for the taxineous affinities

[15] PRIANISCHNIKOW, D., Zur physiologischen Characteristik der Ammoniumsalze. Ber. Deutsch. Bot. Gesell. **26a**:716–724. 1909.

[16] Ber. Deutsch. Bot. Gesell. **27**:582–588. 1909.

[17] NATHORST, A. G., Paleobotanische Mitteilungen. 7. Handl. Kgl. Svensk. Vetensk.-Akad. **43**: no. 8. 1909.

of *Stachytaxus*, and by implication for the similar relationship of *Palissya*. The only real evidence for the affinity of these two genera with the Taxineae seems to rest on the possible presence of an epimatium in connection with the seeds. It seems not improbable that they are really representatives of an araucarian stock different from any now in existence. Some of the later mesozoic Araucarineae possess both the biovulate cone scale and the flaring integument of the genera under discussion. Present indications are that all the mesozoic conifers will ultimately be arranged either under the Abietineae or the Araucarineae in the broader sense.—E. C. JEFFREY.

Orchid flowers and formative stimuli.—FITTING'S work on the effect of pollination and other stimuli upon the postfloration behavior of orchid flowers has been reviewed in this journal.[18] In a second paper,[19] he gives an account of further experimentation of the same kind, and concludes that the changes induced in the perianth, gynostemium, and ovary are at most six, namely: (1) shortening of the life of the perianth, (2) lengthening of the life of the perianth, (3) closing of the flower, (4) swelling of the ovary and gynostemium, (5) fading of the perianth, (6) greening of the ovary and perianth. Each of these may result separately or with several others, in various combinations. Although it seems probable that the influence of the pollen is due to a chemical substance soluble in water and alcohol, FITTING was unable to isolate it in pure form or to identify it. It was determined, by extracting pollen of *Cattleya Trianaei* with water and hot alcohol, that this chemical substance is not found inside the pollen grain, but merely adheres to it and can be removed without injury to the pollen. By using the pollen from which this substance has been removed, the effects of the pollen tube alone can be studied, when it is found that the tube produces the same results as the active substance. This is not due to the substance secreted by the tube or carried down from the pollen grain, but to an unknown factor.—R. CATLIN ROSE.

A new case of apogamy.—*Burmannia coelestis*, as described by ERNST,[20] furnishes a case of apogamy somewhat different from any hitherto reported. From the cells of the egg apparatus of an eight-nucleate embryo sac with diploid nuclei, one and often two and sometimes three embryos are produced. The formation of a tetrad of megaspores is either irregular or completely suppressed, as is already known to be the case in most apogamous forms previously described. No synapsis stage or heterotypic mitosis was observed. The number of chromosomes was not determined, but is greater than in normally fertilized species of *Burmannia*. The anticipated irregularities in the pollen were found, and the

[18] BOT. GAZETTE **47**:479. 1909.

[19] FITTING, H., Weitere entwickelungsphysiologische Untersuchungen an Orchideenblüten. Zeitschr. Bot. **2**:225–267. 1910.

[20] ERNST, A., Apogamie bei *Burmannia coelestis* Don. Ber. Deutsch. Bot Gesell. **27**:157–168. *pl.* 7. 1909.

fact that fertilization is very easily demonstrated in normally fertilized species makes the writer confident that the failure to find it in *B. coelestis* is evidence that it does not occur. The figures show only topography, without any details of the chromatin situation. A careful counting of chromosomes at critical stages, and a few figures at the stages which show whether a form is apogamous or not, would have extended the paper but little, and would have made unnecessary any further work upon the subject; but as it is, a forthcoming paper will deal with these details, the present one being preliminary.—CHARLES J. CHAMBERLAIN.

Spermatogenesis in Mnium.—As a result of their studies of several species of mosses, the Drs. VAN LEEUWEN-REIJNVAAN reported that in the last division of the spermatogenous cells a second numerical reduction of chromosomes takes place. In a species of *Mnium* having eight chromosomes in the last division, two long and two short chromosomes pass to the daughter cells. WILSON,[21] studying *Mnium hornum*, in a preliminary note announces that no such reduction is found, and that the gametophyte number is constant throughout spermatogenesis. The resting nucleus before the final division is quite large and contains a small nucleolus. A continuous spirem is not present, and the chromatic material appears as a number of small masses from which the chromosomes are formed. In the final division the axis coincides with the long axis of the cell, there being no diagonal division. Six chromosomes can easily be distinguished in the last division, and it is clear that no such reduction as described by the Drs. VAN LEEUWEN-REIJNVAAN takes place in *Mnium hornum*.

It is to be hoped that the final paper will also deal with fertilization, for many investigators find some difficulty in accepting the account given by the Drs. VAN LEEUWEN-REIJNVAAN.—W. J. G. LAND.

Hydrogen bacteria.—The epoch-making researches of WINOGRADSKI (1887–) on the sulfur, nitrite, and nitrate bacteria established the important fact of the existence of non-chlorophyll organisms that are obliged to manufacture their organic food by energy obtained from the oxidation of various simple inorganic substances. In 1906 various investigators reported the existence of bacteria that can oxidize hydrogen as the source of energy for assimilating CO_2. The forms were shown to be capable of using organic food as well, and are therefore facultatively autotrophic, in contrast to the obligate autotrophic forms studied by WINOGRADSKI. LEBEDEFF[22] now makes a preliminary report of the main results of an extensive study of the metabolism of these forms. The fixing of 100 c.c. of CO_2 requires the oxidation of 500–1500 c.c. of H_2. The oxygen for the process is best obtained from atmospheric oxygen, but in absence of it nitrates can be decomposed as its source. The oxidation of H_2 still continues in the presence of organic food, but no CO_2 is fixed in that case.—WILLIAM CROCKER.

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[21] WILSON, M., Preliminary note on the spermatogenesis of *Mnium hornum*. Annals of Botany **24**:235. 1910.

[22] LEBEDEFF, A. J., Ueber die Assimilation des Kohlenstoffes bei Wasserstoff oxydierenden Bakterien. Ber. Deutsch. Bot. Gesell. **27**:598–602. 1910.

Proteases.—VINES,[23] in continuing his work on the proteolytic enzymes of plants, finds that both malt extract and taka-diastase (Parke Davis and Co.) contain enzymes capable of digesting fibrin and of splitting peptone. From malt extract he has isolated the peptone-splitting enzyme free from the fibrin-digesting body, and from taka-diastase he separated each from the other. Both these enzymes seem to act best in acid media. In animal tissues there are two fibrin-splitting enzymes: α protease, weak and acting in basic media; and β protease, more powerful and acting in acid media. By special methods of preparation, VINES obtained a protease which acted best in neutral and basic media. This perhaps corresponds to the α protease of animal tissues. The ereptases, peptone-splitting enzymes, of animal tissues act best in basic media. VINES's work shows that plant ereptases act in acid media. As to terminology, one is inclined to believe that VINES could adopt profitably that of animal workers as given by VERNON.[24]—WILLIAM CROCKER.

A new genus of Cordaitales.—SCOTT and MASLEN[25] have described a new genus (*Mesoxylon*) of Cordaitales from the calcareous nodules of the Lower Coal-measures of Lancashire. It is intermediate between *Poroxylon* and *Cordaites*, as its name implies, including five species which have been referred heretofore to these two genera. The combination of characters is the anatomical habit of *Cordaites* and the centripetal xylem of *Poroxylon*. The pith is relatively large and discoid (as in *Cordaites*); the wood is dense, with narrow pith rays and relatively small tracheids; the leaf traces are double, but divide before entering the leaf; the centripetal xylem is present in the leaf traces at the margin of the pith (as in *Poroxylon*) and throughout their course to the leaves. The genus is thought "to completely bridge the gap, so far as anatomy is concerned, between the Poroxyleae and the Cordaiteae," and helps to connect the cordaitean and later forms (excepting cycadophytes) with the "pteridosperms."—J. M. C.

"Bars of Sanio" in Coniferales.—The "bars of Sanio" are "folds" of cellulose to be observed in the walls of tracheids as horizontal or more or less semicircular markings, which stand out clearly with proper staining. Miss GERRY[26] has investigated their distribution among the Coniferales, and has discovered that they furnish a constant and useful character in the determination of fossil woods. They were found in 35 of the living genera, but do not occur in *Agathis* and *Araucaria*, nor in the mesozoic araucarians. Since they do occur in the podocarps, it is concluded that this group is more closely related to the Abietineae than to the Araucarineae, a conclusion which contradicts a growing conviction based

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[23] VINES, S. H., Proteases of plants. Annals of Botany **24**:213–222. 1910.

[24] VERNON, H. M., Intracellular enzymes. London: John Murray. 1908.

[25] SCOTT, D. H., and MASLEN, A. J., On *Mesoxylon*, a new genus of Cordaitales (preliminary note). Annals of Botany **24**:236–239. 1910.

[26] GERRY, ELOISE, The distribution of the "bars of Sanio" in the Coniferales. Annals of Botany **24**:119–124. *pl. 13*. 1910.

on other characters. It is an interesting fact that the ancient *Prepinus* shows these "bars," which fact helps to establish their ancient character. The total result is to emphasize strongly the distinctness of the araucarians from all the other Coniferales.—J. M. C.

Embryo sacs of some Onagraceae.—An investigation[27] of *Epilobium angustifolium*, *E. Dodonaei*, *Oenothera biennis*, and *Circaea lutetiana* shows an interesting variation from the conventional development of the embryo sac. The usual tetrad of four megaspores is formed and the lowest one enlarges and begins to develop in the well-known way, but as soon as the four-nucleate stage is reached, two synergids and an egg are formed at the micropylar end of the sac, leaving one free nucleus in the middle or toward the antipodal end. This sac looks like that of *Cypripedium*, as described by Miss PACE,[28] but is formed from one megaspore, while that of *Cypripedium* is formed from two. At fertilization, one male nucleus fuses with the nucleus of the egg and the other with the single polar nucleus, so that there is no triple fusion as in *Cypripedium*, where one of the synergids takes part. The embryo and endosperm develop in the usual way.—CHARLES J. CHAMBERLAIN.

The original Oenothera Lamarckiana.—GATES[29] has discovered a manuscript in the Sturtevant collection of the library of the Missouri Botanical Garden "which proves that this plant was originally a species growing wild in Virginia, and that it was the first *Oenothera* introduced into European gardens, about 1614." In view of the fact that the origin of this important species has been in doubt, and that it has been claimed to have originated in cultivation, this discovery is noteworthy. The record referred to is in the form of a long marginal note in a copy of BAUHIN'S *Pinax*, by JOANNIS SNIPPENDALE, and is an accurate description of the plant. "The record is as complete and accurate as could be desired, to prove to one familiar with the characters of these forms the identity of the plants in question." The plant was described under BAUHIN'S name, *Lysimachia lutea corniculata*.—J. M. C.

Color inheritance in Lychnis.—SHULL[30] has discovered that the purple color in *L. dioica* is a compound character, produced by the interaction of three distinct and independent genes. The two types of purple color present in different individuals are a reddish purple, changed to blue by alkalies, and a bluish purple, changed to red by weak acids. The bluish or alkaline color is hypostatic to the reddish or acid color, which is the reverse of the condition found in all other

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[27] MODILEWSKI, J., Zur Embryobildung von einigen Onagraceen. Ber. Deutsch. Bot. Gesell. **27**:287–292. *pl. 13*. 1909.

[28] BOT. GAZETTE **44**:353–374. *pls. 24–27*. 1907.

[29] GATES, R. R., The earliest description of *Oenothera Lamarckiana*. Science N.S. **31**:425, 426. 1910.

[30] SHULL, GEORGE H., Color inheritance in *Lychnis dioica* L. Amer. Nat. **44**:83–91. 1910.

plants containing similar series of colors. It is inferred that crosses between white-flowered plants should result not infrequently in progenies of all purple-flowered offspring, or of purple and white in the ratios 1:1, 3:5, or 1:3; but as yet these results have not been found.—J. M. C.

Jurassic flora of Normandy.—LIGNIER[31] has added a number of new species to the rich jurassic flora of Normandy, that are suggestive of relationships concerning which real knowledge is very much desired. The Filicales are represented by species of *Lomatopteris* and *Linopteris*, and the Equisetales by a species of *Equisetites*. The cycadean forms, however, are of chief interest and abundance, and it would be a great gain to know definitely what the numerous species of *Zamites* and *Otozamites* represent. The conifers are represented by species of *Brachyphyllum*, *Pachyphyllum*, and *Conites*.

The memoir is undated, but its reception in March 1910 suggests recent publication.—J. M. C.

Apospory and apogamy in Trichomanes.—GEORGEVITCH[32] has investigated *Trichomanes Kaulfussii*, whose apospory and gemma production was described by BOWER in 1894. The branching filamentous prothallium bears sterigmata (singly or in tufts), at the ends of each of which is balanced a gemma. The development of prothallia from these gemmae is described in detail, and antheridia were observed developing directly upon the gemmae, sometimes associated with a prothallium on the same gemma. This transition from sporophyte to gametophyte is accompanied by no reduction in the number of chromosomes. Counts were made in both generations and at different stages of mitosis, and always approximated 80.—J. M. C.

Parasitic fungi of Wisconsin.—In 1884 TRELEASE published a list of the parasitic fungi of Wisconsin, and supplementary lists were issued by DAVIS in 1893, 1897, and 1903. Now a fourth supplementary list has appeared.[33] It contains a list of 76 forms occurring on hosts not previously recorded; and 113 forms not reported heretofore from the state. The latter list includes 9 new species and varieties in the following genera: *Ascochyta*, *Cercospora*, *Cylindrosporium* (2), *Gloeosporium*, *Phyllosticta* (2), *Ramularia*, and *Septoria*. This record in reference to 189 forms indicates what interest and persistence can do for any area.—J. M. C.

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[31] LIGNIER, OCTAVE, Végétaux fossiles de Normandie. VI. Flore jurassique de Mamers (Sarthe). Mém. Soc. Linn. Normandie **24**: pp. 48. *pls. 2. figs. 7.* (Undated.)

[32] GEORGEVITCH, PETER, Preliminary note on apospory and apogamy in *Trichomanes Kaulfussii* Hk. et Grew. Annals of Botany **24**:233, 234. *figs. 7.* 1910.

[33] DAVIS, J. J., Fourth supplementary list of parasitic fungi of Wisconsin. Trans. Wis. Acad. Sci. **16**:739–772. 1909.